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SYSTEMS ANALYSIS OF METHODS FOR MEASURING TRACE DISSOLVED ORGANIC MATTER IN SEAWATER

George Francis Diehl

United States
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THESIS

SYSTEMS ANALYSIS OF METHODS
FOR MEASURING TRACE DISSOLVED
ORGANIC MATTER IN SEAWATER

by

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Thesis Advisor: E.D. Traganza

June 1971

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Systems Analysis of Methods for Measuring Trace Dissolved Organic Matter in Seawater

by

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Submitted in partial fulfillment of the requirements for the degree of

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ABSTRACT

The diffuse field of marine analytical organic chemistry is systematically analyzed to select schemes for measuring the trace amounts of dissolved organic compounds which can be adopted as standard, rapid, routine tools to advance the oceanographic understanding of this important aspect of the ocean.

The many methods considered for measuring dissolved organic compounds in seawater have been systematically reduced to three systems that are potentially routine and rapid for shipboard work. These include quantitative gas chromatographic analysis of all amino acids and qualitative results for histidine, cystine, tryptophan, and arginine; gas chromatographic analysis of lipids including light hydrocarbons, fatty acids, and sterols, and; autoanalysis with tetrazoleum blue after charcoal adsorption for soluble sugar compounds like glucose, sucrose, and fructose.

Pumping systems or glass samplers on a chemically inert hydrographic wire followed by pre-centrifugation and inverse multiple filtration in combination with a selected analytical scheme will provide a system for routine analysis of seawater. Synoptic data of these compounds can be compared with total organic carbon which is currently the only routine analysis for organics in seawater.

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I. INTRODUCTION

The presence of trace amounts of organic matter in seawater is well documented, but their importance is not as well known [Vallentyne 1957; Collier 1953; Wangersky 1965; Kalle 1965; Wagner 1969]. Their minute concentrations have defied routine analysis and prevented an understanding of their role in oceanography. Only recently has it been recognized that these dissolved organic compounds account for important physical, as well as biological and chemical differences between natural seawater, and salt solutions of the same mineral composition [Table I]. In order to determine the precise role these organics play, so that they may be used to predict and understand oceanic processes, reliable, routine systems to identify these dissolved species are essential. A main objective of this thesis is to systematically select current techniques which will make it possible for the chemical oceanographer to place less emphasis on analytical chemistry and more on a study of the ocean.

Dissolved organic matter is operationally defined as that organic substance passing through a filter with a pore size of 0.45 microns, under laboratory conditions. Because of this small size, and the fact that on the average, every gram of organic matter is dwarfed by 36,000 grams of salts in 900,000 grams of water, the essence of the problem is to remove the relatively enormous amount of salts, while

TABLE I

PROPERTIES OF SEAWATER AND OCEANOGRAPHIC PROCESSES THAT ARE INFLUENCED BY DISSOLVED ORGANIC MATTER

BIOLOGICAL

1. Energy Sources [Johannes, et al. 1969]
2. Growth stimulators, e.g., vitamins [Johnston 1955]
3. Organic toxins [Hood, et al. 1960]
4. Physiological processes of organisms [Provasoli 1963]
5. Distribution of bioacoustic properties [E.D. Traganza, personal communication]
6. Pollutants and contaminants [Goldberg 1970]
7. Photosynthesis, metabolism, mineralization [Wagner 1969]

CHEMICAL

1. Interaction in calcium carbonate system [Chave and Suess 1970]
2. Interaction in nutrient cycle [Menzel and Ryther 1970]
3. Fluorescence [Traganza 1969]
4. Ion-exchange properties of clays [Duursma 1970]
5. Interaction in CO_2 system
6. Synthesis of marine humus [Kalle 1966]

PHYSICAL

1. Water mass characterization [Blumer 1970]
2. Sea slicks and surface viscosity [Jarvis, et al. 1967]
3. Surface tension [Lumby and Folkard 1956]
4. Color [Christman 1970]
5. Sound transmission [Hood 1966]
6. Heat balance at air-sea interface [Hill 1962]
7. Foaming properties [Garrett 1967]
8. Surface potential [Jarvis 1965]

GEOLOGICAL

1. Formation of petroleum in marine sediments [Slowey, et al. 1962]
2. Properties and composition of sediments [Degens, et al. 1964]
3. Radiocarbon dating [Emery 1960]
4. Occurrence in ferromanganese minerals on sea floor [Graham 1959]
5. Dating by pigments [Vallentyne 1957]
6. Natural light and heavy hydrocarbon seepage from oil bearing submarine sediments

concentrating the minute amount of organic matter to reasonably measurable quantities.

Many concentration and desalting procedures have been proposed to separate classes of compounds from "sea salts." These techniques are scattered throughout the literature in oceanographic, chemical, engineering, and geological journals. Many of these incorporate reactions or procedures which are specific for particular compounds, while others give positive results for distinct molecular groupings. Some methods have been completely worked out; in others, only preliminary steps have been attempted. Each of the published papers contributes only relatively small pieces of information to the organic puzzle. No real effort has been made to step back and examine what progress nas been made in this field, and into which areas to direct future work. Such an approach at this point will be very valuable.

The analysis of organic matter in seawater can be thought of as a system of interconnected procedures. As compound isolation and identification methods are improved, particular techniques stand out as superior in various ways. This becomes evident by their successful use in field and laboratory work. At the same time, other methods seem to have no redeeming value in the light of more sophisticated, accurate, and practical procedures. A careful examination of the advantages and disadvantages of each technique, and of their precision, sensitivity, and selectivity will be valuable to

future investigators for optimizing systems for trace organic analysis in the sea.

In the sequence of organic compound identification one must start with accurate sampling, followed by nondestructive filtration or centrifugation, concentration and/or desalting which must not alter the samples, and finally, quantitative determination of the individual species present. Laboratory procedures and handling often will cause degradation or alteration of the dissolved species, which are indigenous to the very dilute, natural marine environment. In addition to this inherent source of erroneous results, there is the obsequious contamination from such externals as samplers, sampler handlers, filters, reagents, and so forth.

This paper is directed to the search for routine organic analysis and to the need for a more consolidated effort to optimize the procedures for measuring dissolved organic matter. The results of this work will be used at the Naval Postgraduate School to focus on the "ideal" compound, or compounds, which are relevant, relatively easy to measure, and are variable in the ocean. Data on this or these compounds' presence and concentration will be complemented with a total dissolved carbon method to study the marine environment.

A. DISSOLVED ORGANIC COMPOUNDS PRESENT IN SEAWATER

There are many types of dissolved organic compounds in the ocean. Stumm and Morgan (1970) have concisely tabulated

these naturally occurring substances [Table II]. The distribution of the free constitutents has been instructively presented by Degens (1970) [Figure 1]. This paper will show the results of a systems analysis for soluble monosaccharides, lipids and their derivatives, and free and combined amino acids. These three groups were chosen because they are significant in the ocean, and much has been published on methods to identify these compounds in seawater.

1. Soluble Sugars

Among the life substances, monosaccharides or hexoses are principally derived from planktonic activity in the euphotic zone. They are the hydrolysis products of higher carbohydrates. Knowledge of their presence and concentration can therefore be a valuable measure of the past and present biological activity in an area. Paradoxically, with all methods that have been developed to quantitatively measure individual organic compounds in the sea, the techniques used for monosaccharides have been, in the past, the least numerous, least sensitive, least accurate, and least reliable. This was due, in part, to the fact that monosaccharides are especially susceptible to bacterial digestion, decomposition, and rearrangement during concentration and desalting procedures. Also, monosaccharides are unstable, very reactive organic compounds which may affect the diagentic processes [Josefsson 1970]. However, there is now a good deal of optimism for improving these methods to the point where development of a routine analysis is likely.

TABLE II

Naturally Occurring Organic Substances

Life Substances	Decomposition Intermediates	Intermediates and Products Typically Found in Nonpolluted Natural Waters
Proteins	Polypeptides → $RCH(NH_2)COOH$ (amino acids) → $RCOOH$, $RCH_2OHCOOH$, RCH_2OH, RCH_3, RCH_2NH_2	NH_4^+, CO_2, HS^-, CH_4, HPO_4^{2-}, peptides, amino acids, urea, phenols, indole, fatty acids, mercaptans
Polynucleotides	Nucleotides → purine and pyrimidine bases	
Lipids		
Fats, Waxes, Oils	$RCH_2CH_2COOH + CH_2OHCHOHCH_2OH$ (fatty acids, glycerol) → RCH_2OH, $RCOOH$, shorter chain acids, RCH_3, RH	CO_2, CH_4, aliphatic acids, acetic, lactic, citric, glycolic, malic, palmitic, stearic, oleic acids, carbohydrates, hydrocarbons
Carbohydrates		
Cellulose, Starch, Hemicellulose	$C_x(H_2O)_y$ → monosaccharides, oligosaccharides, chitin → hexoses, pentoses, glucosamine	HPO_4^{2-}, CO_2, CH_4, glucose, fructose, galactose, arabinose, ribose, xylose
Lignin	$(C_2H_2O)_x$ → unsaturated aromatic alcohols → polyhydroxy carboxylic acids	
Porphyrines and Plant Pigments		
Chlorophyll, Hemin, Carotenes and Xantophylls	Chlorin → pheophytin → hydrocarbons	Pristane, carotenoids
Complex Substances Formed from Breakdown Intermediates, e.g.,		
	Phenols + quinones + amino compounds →	Melanins, melanoidin, gelbshoffe
	Amino compounds + breakdown products of carbohydrates →	Humic acids, fulvic acids, "tannic" substances

(from Stumm, W., and J.J. Morgan, 1970.)

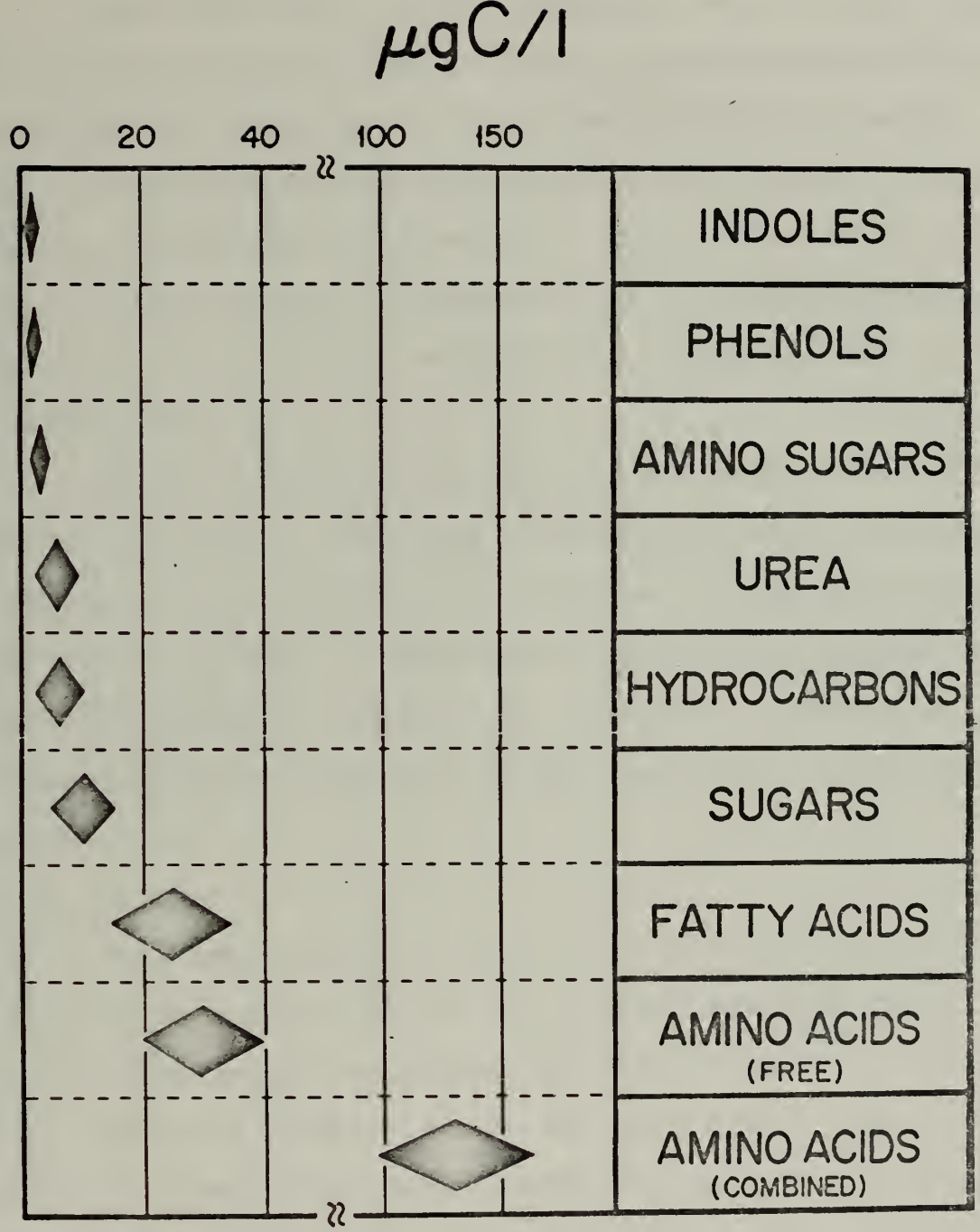


FIGURE 1: Distribution of free constituents in sea water. The individual samples have been grouped into systematic classes of compounds and have been plotted in the form of cumulative frequency diagrams to summarize the information in a comprehensive form. The diamond-shaped figures represent the 2 sigma range. The data are presented in μg C/liter to allow a direct comparison to the total dissolved organic matter which is generally reported in mg C/liter. (Degens, E.T., 1970)

2. Amino Acids

Amino acids are another important water soluble hydrolysis product, derived from proteins. They have been found to be biologically important to marine ecology in many ways. These include excretion by plankton and utilization by algae, bacteria, and marine invertebrates. They are essential for growth in some species of phytoplankton by satisfying certain micro-nutrient or vitamin requirements [Shiraishi and Provasali 1959].

Amino acids have been found in all oceans, and are variable with depth. They are continuously being recycled in the marine environment. Flux data as well as concentration levels are keys to understanding community energetics. A reliable method to measure this component is needed to correlate the many biological events and other properties in the sea.

3. Lipids

The class lipids includes compounds that may be chemically unrelated, but are all characterized by their solubility in organic solvents like ethyl acetate or chloroform. They are commonly esters of fatty acids. Their derivatives include hydrocarbons, mono-, di-,and triglycerides, sterol esters, steroids, free fatty acids, and phospholipids.

Lipids have been more extensively examined because they appear to compose from 10-50% of the total dissolved organic carbon in seawater [Jeffrey 1970], and they are relatively easily separated from seawater by liquid

extraction. They have been found in natural surface slicks, deep anoxic waters, and surface waters, in both coastal and oceanic environments. They were found to be directly proportional to the total dissolved organic carbon which in turn is proportional to the distance from land and depth of water [Jeffrey 1970].

The high concentrations of lipids found in sea water result from the fact that they are more resistant to biological attack than amino acids or carbohydrates and because of their relative greater abundance. A knowledge of their concentrations would help determine the natural background of dissolved organics in the oceans, before human pollution becomes extensive.

Hydrocarbons, considered a lipid in this study, have been found in all depths of the seas [Swinnerton and Linnenbom 1967]. Garrett (1967) found that lipids are a primary constitutent of natural sea slicks that alter the physical characteristics of the air-sea interface. In addition, vertical and horizontal fatty acids profiles may be used to study mixing rates and current patterns in the ocean [Slowey, et al. 1962].

A more detailed study of the relationship of lipids to marine organisms can be found in a paper by Lovern (1964). In this valuable paper Lovern discusses the lipids of marine plants, invertebrates, vertebrates, mammals, and their metabolic roles, for example, in marine diets.

II. SAMPLING PROCEDURES

On critical analysis of the methodology for measuring dissolved organic matter in seawater, it becomes apparent that despite the improved analytical techniques for isolating and identifying the trace organic constituents, methods for sampling the seawater have not progressed to the same level of development. In fact, in many papers dealing with the determination of these dissolved species the sampling method is often obscure or undefined. This seems to indicate that perhaps not enough consideration is given to this fundamental step in the whole system. The results of current organic analysis are for the most part dependent on a large, uncontaminated sample that is representative of the seawater desired.

The primary reason for proper sampling procedures is to prevent probable contamination of the seawater sample from the device itself, or during transfer and storage of the sampler. This contamination is effected by a variety of degradation or condensation reactions catalyzed by container walls resulting in loss or modification of the compound sought, or its phase transfer. Furthermore, regarding organic constituents, contamination of even a few orders of magnitude below total organic carbon (generally <1 milligram/liter) is critical relative to the concentrations of the individual component compounds present.

Many seawater sampling devices have been proposed or used. Table III is a compilation of samplers that have either been designed specifically for dissolved organic matter, or samplers that have been modified for this use because of their large volume and potential low contamination. Table III was prepared using certain criterion for the "ideal dissolved organic matter sampler." These criteria are based on those of Clark and his co-workers [1968] and include:

1. minimum contamination
2. large volume sample
3. ease of shipboard handling
4. ease of cleaning and short turnaround time
5. simplicity of design
6. reasonable cost
7. prevention of organic reactions, e.g., oxidation, and
8. ease of transfer and storage.

With respect to the large volume criterion, it must be remembered that there are analyses that require small volumes that may have a better chance of becoming routine for oceanographic studies.

These devices in Table III collect large volume samples in two ways: either by continuous pumping on board, or by collecting descrete samples. They have been designed for surface collection, or at depth. Some samplers may include in situ filtration (see filtration section). There is very little critical comparison of various samplers [Gordon 1969], and it is hoped that Table III will be helpful in listing

Table III: Dissolved Organic Matter Sampling Devices

NAME OF SAMPLER	ANALYSIS FOR WHICH DESIGNED	MATERIALS WITH WHICH SEA WATER COMES IN CONTACT	VOLUME OF SAMPLER	DIMENSIONS OF SAMPLER AND EASE OF HANDLING	AUTHOR	COMMENTS
Rupture-disc triggered sampler	dissolved organic matter	glass and teflon	1-15 l. adjusted by tie rods	137 x 16.5 (O.D.) cm; can be lowered on 0.425 cm. hydrographic wire	Clarke, et. al. (1967)	1. Designed for work at 4500 meters. 2. Allows for evacuation or filling with inert gas to prevent oxidation of sensitive organic compounds. 3. Easily dissembled and can be cleaned ultrasonically. 4. Collected in situ thru a Millipore filter. 5. Glass liner can be removed and stoppered.
"Dazzler"	dissolved organic carbon	glass pipe, with teflon ends, stainless steel operating parts are external	10 l.	designed to be supported singly on the end of a hydrographic wire	Menzel and Ryther (1968)	1. Used regularly in dissolved carbon analysis at WHOI.
Nisken bottles	large volume hydrographic casts	primarily polyvinyl chloride with lucite end plugs and surgical rubber as an internal closing spring	1.7 l.; 5.0 l.; and 30.0 l.	the Nisken Rosette is 18" x 18" (O.D); remote control depth sensor; accomodates reversing thermometers on each bottle	Nisken (1968)	1. Practical and compact. 2. Device can be stored and handled without removing samples.
"Tantalus" bottle	radioactive trace metals and particulate organic matter	plastic milk churns and rubber stoppers	40 l.	can be used on 4 mm hydrographic wire; sampler is easily repaired	Duursma (1967)	1. Cheap and simple in construction. 2. Sampler is hauled up in a horizontal position to reduce water pressure on container. 3. Tested to 2000 m. 4. Young (1969): "... light and cheap, but not rugged enough for routine work."
Beer keg samples	C^{14} analysis	stainless steel	60 l.	simple to operate and rugged; easy to repair; used from a low hydroboom; lightweight and compact.	Young (1969)	1. Satisfactory flushing. 2. Average turn-a-round time on surface is 15 minutes. 3. Tested to 4000 m. 4. Can be cleaned ultrasonically.

Table III: Dissolved Organic Matter Sampling Devices (continued)

NAME OF SAMPLER	ANALYSIS FOR WHICH DESIGNED	MATERIALS WITH WHICH SEA WATER COMES IN CONTACT	VOLUME OF SAMPLER	DIMENSIONS OF SAMPLER AND EASE OF HANDLING	AUTHOR	COMMENTS
Gerard-Ewing sampler	radioisotope analysis	epoxy resin lining; melamine plastic door	220 l.	51 x 18.25 (O.D) in.; equipped with reversing thermometers	Gerard and Ewing (1961)	1. Has means for determining depth of sampler. 2. Flushing is very good. 3. Used routinely by Lamont Geological Observatory.
Multipurpose large volume sampler	C^{14}; trace elements; particulate organic matter, and "potentially for dissolved organic matter."	sampler is lined with trifluoro-chloro-ethylene polymer; neoprene valves.	60 l., 140 l., and 160 l. models	80 x 15 (O.D.) in.; must use 600 lb. load wire, handling must be done very carefully to avoid pre-tripping.	Bodman, et. al. (1961)	1. Efinger (1967) has developed a depth recorder that can indicate sample depth with an accuracy of 5-10% immediately after gear is brought aboard. 2. Relatively expensive. 3. Author comments that slight modification would be necessary for routine DOM work.
Van Dorn water sampler	biological, and C^{14} analysis	plexiglas, surgical tubing and rubber "force cups"	4 and 50 l. models	larger sampler is fastened to end of hydrographic wire; smaller samplers may be lowered in multiples along the wire by wing nuts	Van Dorn (1956)	1. Free-flushing. 2. Messenger activated.
Bag sampler	silicon-32 analysis, recommended for DOM upon modification to prevent contamination	bag is a hypalon-coated nylon fabric, reinforced by nylon webbing and rubber	"30 tons" (7.26×10^5 l.)	the bag is 42' long when opened; 20-30 m^2 is needed to ready sampler; a 1800 kg wench is needed	Schink and Anderson (1969)	1. Divers may be needed to orient bag in water. 2. Pinger on the cable is used to determine the height of sampler. 3. Sample is pumped on-board from 20 m. below the surface.
Screen technique for surface layers	dissolved organic matter	Monel metal	approximately 100 ml. per dip; 20-l. sample required 200-250 surface contacts over a 90-110 m^2 area	a mesh area (60.2% open space) 75 x 60 cm. is dipped into the surface	Garrett (1965)	1. Only 75% efficient due to initial adsorption of a portion of monolayer onto the screen. 2. Vertical mixing and surface contamination by seaweed and copepods are problems. 3. Harvey (1969) "... time required to process large samples was excessive."

Table III: Dissolved Organic Matter Sampling Devices (continued)

NAME OF SAMPLER	ANALYSIS FOR WHICH DESIGNED	MATERIALS WITH WHICH SEA WATER COMES IN CONTACT	VOLUME OF SAMPLER	DIMENSIONS OF SAMPLER AND EASE OF HANDLING	AUTHOR	COMMENTS
Surface micro-layer collector	organic matter in upper 60μ of sea water surface	neoprene, ceramic coating, and a poly-ethylene catch bottle	20 l.	cylinder is 39 cm (O.D) x 60 cm and is pushed ahead of small boat to collect layers at a rate of "hundreds of m^2/hr."	Harvey (1966)	1. Thickness of layer collected depends on speed of cylinder rotation. 2. Causes a minimum of vertical mixing. 3. Same cleaning procedures as with glass and plastic. 4. Reed (1969) modified this skimmer apparatus.
Biodialystat	dissolved organic matter and vitamins in lake water	Pyrex glass and silicon (gaskets)	50 ml. (large volume biodialystats are possible as long as the membrane surface/volume ratio is maintained	only tested to a depth of 30 m.	Parker (1967)	1. Operates on the principles of solute diffusion. 2. Biodialystat must be calibrated for each compound sampled. 3. Samples in biodialystats remain sterile for at least 96 hours and can be easily cleaned in autoclave. 4. Parker [personal communication] believe there is no reason it can't work in the marine environment.
Submerged membrane filter sampler	marine bacteria	316-Stainless steel	variable volume, up to 1.5 l.	handling is kept to minimum; 603 x 78 mm; opening and closing are not subject to mechanical failure	Williams (1969)	1. Tested to 6000 m. 2. Designed for work @1000 meters. 3. Filters <u>in situ</u>.
Submersible batch filtering unit	particulate organic matter (which can be converted for DOM work)	stainless steel or teflon	15 l./min.	consists of a pump, filter, hose, and gage unit	Laird, et. al. (1967)	1. Pump has magnetic drive (no shaft seal required). 2. Used up to 200 m and greater. 3. Unit can operate in wide degrees of clogging. 4. Unit is equipped with membrane filter, which filters <u>in situ</u>.
Submersible sampling pump	chemical, physical and biological analysis	brass and stainless steel; polyvinyl chloride	3 or 4 l./min	centrifugal pump coupled to a submersible electric motor	Whaley (1958)	1. Filter can be installed. 2. Especially useful when corollating with dissolved O_2, i.e., it is bubble free. 3. Pumped directly on board from 200 ft.

Table III: Dissolved Organic Matter Sampling Devices (continued)

NAME OF SAMPLER	ANALYSIS FOR WHICH DESIGNED	MATERIALS WITH WHICH SEA WATER COMES IN CONTACT	VOLUME OF SAMPLER	DIMENSIONS OF SAMPLER AND EASE OF HANDLING	AUTHOR	COMMENTS
Continuous pumping extraction system (1963)	dissolved organic matter (lipids)	linear polyethylene tubing	8-12 l./min.	may be pumped from any depth by a deck mounted 1-hp jet pump	Zeitoun, et. al. (1965)	1. The flow passes a 0.45μ Millipore filter, and into an extraction system. 2. Used at 3500 m and shallower. 3. This system is the basis of other continuous systems later used by L.M. Jeffrey and D.W. Hood.

alternatives to solving the contamination-sampling problem. In this regard, a standard sampling procedure is preferable and may be obtained by utilizing the desirable aspects of each of the samplers listed. An ideal sampler for universal usage may be a possible outcome of this study, similar to the results of Clark and his co-workers (1968).

A. MATERIALS

Most investigators in this field will insist that glass is the best material for collecting uncontaminated samples, but glass is expensive and a difficult material to use in constructing samplers. Metal containers are not often used, except for stainless steel, due to impurities and also reactivity with organics. Some plastics emit soluble plasticizers into a sample and thus contaminate it. It has been shown that such commercially used plasticizers as the phthalite esters bleed out of container walls during storage or processing [Nishiwaki and Fukai 1970]. Jeffrey (1970) recommends polyvinyl chloride because it is less expensive, allowing a more universal standard usage, and that any contamination from this plastic by soluble plasticizers that is possible, is apparently at a very low and acceptable level. In most cases, the proper procedure before using any material in a sampler is to examine its specific interference with the organic compound or compounds under study.

B. SAMPLING DEVICES

If synoptic profiles are desired, large multiple quantities of water must be collected. This implies the Nansen bottle type casts, but more commonly a single sampler fastened to the end of a hydrographic wire. The resulting collection and shipboard filtration becomes a slow and extremely cumbersome task. Several investigators have overcome this problem by pumping seawater samples from depth and, in some cases, subsequently through a filter, and adsorption or extraction unit, in one continuous operation. This technique provides large volume samples up to 9000 liters providing three milligrams of organics [Jeffrey 1969]. This procedure also eliminates the extra handling involved in filtering, transfer, and storage. However, attention must be given to contamination by gain or loss of dissolved compounds in the tubing, and the rupture of biological cells by the pumping technique. Also, it should be noted that with deep water pumps, submersible pumps have the advantage that they take in samples at the desired inlet at the end of the tubing, and are bubble-free; whereas deck-operated pumps may take in "samples" from any opening or leak along the entire length of the pump's tubing and are unsuitable for gas-free sampling. Nevertheless, recently, more and more investigators are using submersible pumping (and filtration) units in their study of dissolved organics [Jeffrey 1969; G.W. Harvey, personal communication]. It is not clear whether this technique is wholly satisfactory with respect to contamination, or how much mixing

takes place in the line, but it is potentially very valuable mainly because it automates the analysis. Woods Hole Oceanographic Institute [Dr. Blumer and Dr. Edhardt, personal communication] is pumping from 4 depths with 4 pumps at each depth, to obtain synoptic replicate profiles.

The sea surface contains higher concentrations of dissolved organic matter than other positions in the water column [Garrett 1970]. It is an area of increased microbial activity [Williams 1967] and a possible significant site of photochemical reactions [E.D. Traganza, personal communication]. Collection of seawater samples in this microenvironment is vital. Organic matter, especially polar and water insoluble species, show sharp concentration gradients in these layers. The collection problem here is to obtain representative samples from a thickness from one centimeter to several Angstroms. Garrett (1965) and Harvey (1966) and others not listed in Table III [Goering and Menzel 1965, 1967] have proposed samplers to do this. Although much of the early work on sea slicks [Jarvis 1967, and Williams 1967] have been done using Garrett's sampler, low efficiency with respect to total collection of materials present, due to adsorption on the Monel metal collecting screen, vertical mixing from below, and floating surface contaminates, all contribute to give only qualitative data, at best.

Harvey's rotating cylinder apparatus seems to avoid these problems and also makes it possible to sample a much larger

sea-surface area more rapidly and to collect layers as thin as 60 microns. James Reed (1969) has successfully used a modified Harvey "skimmer" to collect lipoid substances from the surface film of "windrows" in Monterey Bay, California.

C. QUALITY CONTROL

Quality control is an important aspect of accurate sampling that must be borne in mind no matter which sampling technique or device is used. Bowen [unpublished] and Hood (1968) have established quality criterion analysis for samples which are collected individually or continuously, respectively. Generally, measurement of any chemical parameter that has established or easily measured concentration gradients should complement any hydrocast to assure that the sample is properly associated in the synoptic picture of the ocean. Actual sample depth, and recorded sample depth as indicated by the depth at which the tripping mechanism was activated are frequently not the same. This is especially important in the case of the very large volume samples [Schink and Anderson 1969]. Such chemical properties as salinity, dissolved oxygen, silicate, and phosphate have been used to verify the organic concentrations. This is normally done by comparing consecutive organic subsamples for agreement with the known or established concentrations profiles of a chemical parameter at each hydrostation. This procedure has not been used in the majority of the papers reporting organic matter concentrations in seawater and is possibly a major

reason for the lack of agreement on many organic profiles published by various authors.

III. STORAGE AND CLEANING OF APPARATUS

Once a large water sample is collected for subsequent dissolved organic analysis, it must either be processed immediately, or preserved and stored for analysis in the institutional laboratory ashore. If sampled properly, the task of immediately processing the water collected follows directly, assuming that all glassware and materials that come in contact with seawater are scrupulously clean. If it is necessary to store the sample for future work, several precautions must be taken. During storage, the quality of the sample may change due to adsorption of organics or detritus onto container walls, and to microbial degradation or utilization. The former problem is solved by following the same criteria for choosing the type of material which is suitable for sampler construction. The latter problem has been resolved to varying degrees by the use of acidification [Webb and Wood 1967], chloroform [Chau and Riley 1966], $HgCl_2$ [Josefsson 1970], thymol [Palmork 1963b], and deep-freezing of the sample [Degens, et al. 1964]. These procedures, although used to prevent bacterial activity, may also kill organisms, releasing their cellular components [Webb and Wood 1967; Gilmartin 1967]. Parker (1967a) suggests that if immediate analysis is not practical, then freezing is

permitted. However, while stored at low temperatures, adsorption of volatile organics, and CO_2 fixation by contaminating micro-organisms may be possible sources of additional organic matter. In general, any method of attenuating microbiological activity should be examined carefully to avoid creating additional sources of experimental error.

In all micro-organic analysis, ultra clean chemical apparatus is vital. This is especially true when working in the parts per billion organic concentrations found in seawater. If possible, work should be done in a "clean" room to prevent contamination from such unsuspected sources as room air vents [Blumer, personal communication]. Such substances as stopcock grease and impure solvents should never be used. Glass and plastic equipment must be thoroughly cleansed of all possible contimination. Dr. Jeffrey [personal communication] has recommended a scheme which she uses in all trace organic work. In it, polyvinyl chloride is cleaned initially with soap and water, then rinsed with "clean" ethyl alcohol and again with acetone. As for cleaning glassware, a good scrubbing with soap and water is done initially, followed by distilled water rinses. The glass is then soaked in good cleaning solution (chromic acid and H_2SO_4), then rinsed with distilled water. To remove any traces of insoluble grease that the cleaning solution may not have oxidized, rinses with acetone, chloroform, and alcohol are adequate. After drying, the glassware should be covered with aluminum foil. If samples of less than a liter

are processed, it is worth the time to finally rinse with organic-free distilled water. This can be obtained by putting 5 grams/liter of potassium persulfate in distilled water and leaving it overnight, or by ultraviolet radiation of water containing 1 ml/liter of H_2O_2 [Jeffrey, personal communication].

When glassware is not in use, it should be covered, and then cleaned just before use. Another alternative is to store it in distilled water containing 5 gm/liter of persulfate solution.

IV. FILTRATION PROCEDURES

It is evident from Tables IV,V, and VII and the results of Parker (1967a) that many investigators fail to describe the details of their filtering procedures. Since many artifacts may result from improper filtering techniques, meaningful comparison of results of various authors is not possible unless either a standard procedure is adopted, or the filtering step is described completely. That is, the type of filtration should be specified including the type of membrane used, pretreatment, pore size, amount of positive or negative pressure, filter surface area and volume filtered. Parker (1967a) has presented a valuable comparative study of various methods of filtering natural waters.

The principal reason for filtering seawater samples is to obtain the dissolved organic fraction free of plankton, particulate matter, and bacteria. The filtrate of seawater,

passed through a filter of 0.45 microns pore size is commonly considered to contain only the dissolved organic fraction. However, Ogura (1970) has found that dissolved organic particles, less than 0.45 microns are sometimes retained on a 0.45 micron filter by adsorption onto the surfaces of inorganic and organic particulate matter that are held back by the filter. This indicates that perhaps a group of dissolved compounds that may be susceptible to adsorption may never have been detected. Garrett (1967) realized this phenomena and did not filter his sample before analyzing for organics at the sea-surface interface. Ogura (1970) also indicates that further fractionation of the dissolved species is possible. This may be a useful tool to isolate the dissolved compounds by molecular size. For example, he found that in a Scenedesmus suspension, filtration through filters of decreasing pore size resulted in the following breakdown:

SIZE RANGE (MICRONS)	% CONCENTRATION OF DISSOLVED ORGANIC CARBON
0.45 - 0.22	8
0.22 - 0.10	4
0.10	88

Parker (1967a) found that although a 0.45 micron pore filter does not remove all bacteria, a small pore size (viz. 0.22 micron) may trap hydrated macromolecules that are part of the dissolved fraction. Johannes (1968) noted that the few

bacteria that are allowed to pass a 0.45 micron pore filter are not enough to cause problems for the first couple of hours.

Cellulose ester membranes [Millipore, Gelman, Schleicher and Schuell Companies] are the most commonly used filters in the field despite some of their inherent pitfalls. Glass fiber filters and metal fiber filters, such as silver, are being used more often. Membrane filters can be washed, are readily available, and allow a reasonable flow rate. However, they contain 2 to 3% of their dry weight as detergents such as Triton X-100 [Chan 1967]. In addition to the contamination, this causes a foam in the filtrate if not completely washed, and can cause cell rupture. Such interference may confuse the investigator, as reported by Wallace and Wilson (1969) and result in erroneous results. Furthermore, Guillard and Wangersky (1958) found that unless membrane filters are washed before use, they will elute soluble carbohydrates. In fact, they caution that significant errors will result if samples less than one liter in volume are passed through these filters. This soluble carbon can be removed by passing 60 ml. of 0.1N HCl through the filter [Parker 1967a]. Glass fiber filters owe their increased usage to the fact that they can be efficiently cleaned in an autoclave or by ignition. Parker (1967a) found that, outside of filtration time, there was no significant difference in total carbon produced using a pre-washed 0.45 micron membrane [Millipore] filter, and a 0.45 micron silver fiber

filter [Silas]. The total filtration time in this study was much longer for metal fiber filters, than for membrane filters. Jeffrey claims that Gelman 0.3 micron glass filters are as good as Millipore. They are easily sterilized, and they filter faster because they are thicker and have more surface area [Hood 1968]. Menzel and Vaccaro (1964) recommend filtration with pre-combusted glass fiber filters that have been rinsed with a small amount of sample prior to use. Blumer (1970) claims that clean extracted filter paper, although not as retentive as membranes, eliminates cell injury and allows gravity filtration. In a comparative study, he found that paper filtration and consecutive filtration through paper and clean membrane filters allowed the same materials to pass. He concludes that paper filtration is adequate for qualitative identification of dissolved organics; whereas gravity filtration followed by membrane filtration is desirable for quantitative work.

A. CELL RUPTURE

As more precise methods of analysis are introduced, results are blurred by cellular material rupturing on the filter and falling into the filtrate, increasing apparent concentrations of some organic species. It has been verified that plankton, especially naked flagellates, are subject to cell rupture above a certain, but unknown, pressure drop across the filter [Nishiwaki and Fukvi 1970]. Gentle positive or negative pressures may be used to increase the flow,

but non-destruction of cells must be assured. Many reported values are probably inaccurate because of cell fragments adding to the dissolved content of a filtrate.

One of the reasons for using pressure to promote filter flow, is to overcome the resulting clogging of the pore after passage of water over a period of time. This also, in effect, decreases the pore size allowing only progressively smaller and smaller particles to pass through. A system that would obviate applied pressure, and minimize the clogging limitation is a "cascade" filtering unit. Such a unit, in which the sample is passed through several membrane filters of decreasing pore size, has been used by Lysyj and his co-workers at the U.S. Interior Department's Department of Saline Water (1968). They used a two stage cascade unit, and are planning a multistage assembly. Lewis and Traganza (1971) in their work have used a system of decreasing pore sized fritted glass filters with the added feature of filter inversion to allow gravity to work against the organisms which might clog the filters, i.e., they fall away. Traganza (1969) and Pomeroy and Johannes (1966) used systems based on this principle reported originally by Dodson and Thomas (1964). Since the above problems are eliminated, this will improve the efficiency of each filter for fractionation.

Centrifugation has been used as a pre-filtration step to remove cells that might either rupture on the filter, or clog it. Parker (1967a) found that centrifugation at 6000 x g followed by either membrane or metal fiber filtration

was very efficient in removing seston from seawater samples. Lewis and Traganza (1971) reached the same conclusion and have suggested constructing a large volume, 10-20 liter, centrifuge as a pre-filtration step.

In situ filtration is another solution to the cell rupture problem. Such devices as the "biodialystat" [Parker 1967b], the "rupture disc triggered" sampler [Clark, et al. 1966] and the submerged membrane filter apparatus [Williams 1969] may significantly minimize cell rupture as well as minimize sample handling. This technique has also been applied to submerged sampling-filtering pumps [Spencer and Sachs 1969]. Filtering at ambient pressure should provide a very representative sample of the dissolved organics as they exist in the marine environment. The biodialystat, which is not a filter in the strict sense, operating on the principle of solute diffusion, has shown much potential. According to Parker (1967a), the "biodialystat" is more efficient than filtration. It not only keeps cell injury to a minimum by filtering in situ, but it is designed to prevent filter clogging and also to preserve the sample for 96 hours after collection by sealing and storing in the unit. Although work with the "biodialystat" has been limited primarily to freshwater sampling, Parker [personal communication] believes there is no reason it can not be used in the marine environment. This may be an optimistic view for salts usually kill good ideas conceived for freshwater.

V. ISOLATION PROCEDURES

A. AMINO ACIDS

There has been a relatively large number of papers published on the isolation and measurement of dissolved, free and combined, amino acids. Table IV presents the major contributions for detection, isolation, and analysis of these important compounds in the marine environment. Amino acids have been found in most oceans, bays, estuaries, and gulfs at the surface and at depth. Typical concentration levels that must be detected range from <0.5 μg/liter to 15 μg/liter (ppb). From the data in Table IV, all investigators have filtered their samples to remove particulate matter and bacteria. Most have used 0.45 micron pore-size membranes, while a few, in more recent papers, have used filters with a pore size of both 0.45 micron and 0.30 micron, with no apparent conflicting results with respect to concentration magnitudes. Degens, et al. (1964) filtered with vacuum. High pressure drops have been shown to be a cause of cell rupture which may partly explain his higher concentrations of amino acids. Bacterial decomposition, especially a problem with amino acids, has been inhibited either by freezing, or the addition of chloroform, thymol, or $HgCl_2$, or a combination of a chemical preservative plus freezing.

When all the methods for detecting amino acids are viewed, four fundamental schemes are apparent. After-filtration is

Table IV: Methods of Amino Acid Analysis

AUTHOR & WATER SAMPLED	COMPOUNDS FOUND	FILTRATION METHOD	CONCENTRATION METHOD	DESALT METHOD	MEANS OF IDENTIFICATION	RECOVERY AND/OR SENSITIVITY	COMMENTS
Tatsumoto (1961) surface sea water in Gulf of Mexico, Yucatan Strait, Caribbean Sea, and a British Honduras Reef; Park (1962) deep Gulf of Mexico waters; Park, et. al. (1963) Texas bay waters	approximately 18 free and combined amino acids in acid-hydrolyzates; acid, basic, neutral, and aromatic acids @ 0.5 $\mu g/\ell$ to 13.0 $\mu g/\ell$.	0.45 μ Millipore filter; HgCl was added to prevent bacterial activity	co-precipitation with $Fe(OH)_3$, protein hydrolyzed with HCL. Fe^{++} and cations separated by ion-exchange.	Dowex 50-X8 cation exchange followed by IRA-400 anion exchange column; 96% of amino acids passed were recovered	paper and ion exchange chromatography	maximum recovery is seldom 50% (Chau and Riley, 1966)	1. Ferric oxide as a co-precipatant has low efficiency for neutral and basic amino acid; inferior to that of Al and Ga oxides (Chau, 1966). 2. Concentrations of amino acids in hydrolyzed samples were five times greater than in an identical unhydrolyzed sample. 3. Didn't report that free amino acids were isolated and identified as such. 4. Large sample needed; laborous and time consuming (Palmork 1963a). 5. All studies are only partially quantitative.
Palmork (1963a) sea water	only neutral and aromatic amino acids (gly, thr, val, and phe); no concentrations reported	5-l. sample passed through a 0.45 μ Millipore filter	evaporation after extraction	derivatives were extracted with ether or butanol	circular thin layer chromatography of the dinitro-phenyl-derivatives by adding 2,4-dinitrofluorobenzene to sea water sample	35% recovery (Chau 1966)	1. Palmork states that it is possible to study peptides and proteins in sea water with this technique. 2. Works well in sea water; works well for taurine; suitable automation of the chromatography is needed (Webb, personal communication). 3. Not all amino acids are recovered. 4. Chau and Riley (1966) found recoveries < 40% for alanine and glutamic acid; also derivatives were unstable.

Table IV: Methods of Amino Acid Analysis (continued)

AUTHOR & WATER SAMPLED	COMPOUNDS FOUND	FILTRATION METHOD	CONCENTRATION METHOD	DESALT METHOD	MEANS OF IDENTIFICATION	RECOVERY AND/OR SENSITIVITY	COMMENTS
Palmork (1963b) sea water from approximately 65°N; 7°W	19 free amino acids; acidic, basic, neutral, and aromatic; no concentrations reported	0.45μ Millipore filtration within six hours of collection; treated with thymol to prevent bacterial activity	direct concentration by vacuum evaporation before and after desalting; precipitated salts were filtered off at intervals	Dowex cation exchanger eluted with 0.1 M aq. piperidene	small, circular paper chromatography to separate and identify	90% recovery is claimed	1. Some samples were collected by sucking the sea water through rubber tubing into a carboy by means of suction pump. 2. Concluded, from chromatographic patterns before and after concentration and desalting, that hydrolysis of peptides did not occur (confirmed by Chau and Riley 1966)
Siegel and Degens (1964) surface water samples from Buzzard's Bay	19 free amino acids; acidic, basic, aromatic, and neutral acids; < .28 to 16.25 μg/l.	1 or 2 liter samples were passed through Gelman type A (0.3μ) glass-fiber filter	evaporation with a Buchler Rotary Evapormix after desalting	1 l. sample through Cu-Chelex (iminodiacetate exchange groups) before evaporation	automatic, high pressure ion-exchange chromatography for quantitative results	recovered 100% is claimed	1. Found that bulk of amino acids are in combined form. 2. Low flow rates limit samples to 1 or 2 l. 3. Siegel (1967) describes method and applications in detail. 4. Method is used in estuarine environment by Hall, et. al. (1970).
Schaefer (1964) artificial sea water	only attempted to isolate entire classes of organics	not described	not described	an amphoteric polystyrene, polyacrylate ion-exchange resin that incorporates negative charges within a polymer network of opposite charges (Retardion 11A8)	did not identify individual amino acids	author claims a recovery of 95-100% for amino acids in 50 ml. of artificial sea water	1. Resins are especially efficient with larger molecules like bound amino acids. 2. No resin regeneration is necessary. 3. 90% of inorganic salts are removed with Retardion 11A8 claimed by Schaefer.

Table IV: Methods of Amino Acid Analysis (continued)

AUTHOR & WATER SAMPLED	COMPOUNDS FOUND	FILTRATION METHOD	CONCENTRATION METHOD	DESALT METHOD	MEANS OF IDENTIFICATION	RECOVERY AND/OR SENSITIVITY	COMMENTS
Degens, et. al. (1964) deep ocean water off Southern California ($117^{o}N$, $32^{o}W$)	17 free amino acids; acidic; basic, aromatic, and neutral acids at 16-125 $\mu g/l$.	2 l. samples were frozen; after thaw, filtered through a 0.45 μ filter (Millipore) in vacuuo	filtrate was evaporated to dryness; acidified with HCl and evaporated in vacuuo, again; amino acids were leached from dried salts by 80% EtOH	final desalting was by cation exchange-resins for the dissolved residue	separated by paper chromatography, and identified by visual comparison with amino acid standards	50% recovery (Chau 1966); quantitative estimation of amino acids within 15% was made by visual comparison of the chromatographic spots	1. Quantitative results reported. 2. Freezing sample on collection may have destroyed some cells causing the higher values of free amino acids. 3. Low-recovery because of difficulty in leaching acids from salts Chau and Riley (1966) 4. Combined amino acids were determined by hydrolysis of particulate matter on filter followed by same procedure as above.
Bishop and Louden (1965) Texas bay waters	14 amino acids (no basic acids found) @ 1.0 to 13.2 g/l. (as reported)	5-gal. samples were passed through a glass wool filter, washed with HCL, HNO_3 and distilled water, then filtered through either a 0.8 μ Millipore filter	coprecipatated with $FeCl_3$ and hydrolyzed after filtration. Flash evaporated at $60^{o}C$.	after the evaporated residue was put in solution it was passed over cation exchanger and eluted with NH_4OH	Qualatatively with 1 and 2 dimensional paper chromatography and quantitatively with amino acid analyzer	not given	1. Described procedure would require 2-3 days. 2. Concentrations are higher than previous values of other investigators (may be incorrect concentration units).
Chau and Riley (1966) sea water at approximately $54^{o}N$; $3.5^{o}W$	11 amino acids; acidic, basic, aromatic, and neutral acids @ 2-16 $\mu g/l$.	0.5 μ membrane (Millipore) filter; chloroform was used to inhibit microbial growth	evaporation in vacuuo in two stages (climbing film and rotary-film evaporators)	Amberlite CG120 Cation exchanger column eluted with piperidine	TLC (reproducability 10%); best results are with acid concentrations of 0.5 to 1.0 $\mu g/l$.; repeated ion-exchange on a smaller column eliminated humic acids interfering with TLC	90% recovery is claimed	1. Reliable results by this method are claimed (in Irish Sea). 2. Method is based on Palmork (1963b).

Table IV: Methods of Amino Acid Analysis (continued)

AUTHOR & WATER SAMPLED	COMPOUNDS FOUND	FILTRATION METHOD	CONCENTRATION METHOD	DESALT METHOD	MEANS OF IDENTIFICATION	RECOVERY AND/OR SENSITIVITY	COMMENTS
Webb and Wood (1967) surface sea water in a New York estuary	17 free amino acids found; acidic, basic, aromatic, and neutral acids 0.15 to 11.9 μg/l.	0.45μ Millipore filter; a layer of water is always kept above the filter. The filtered sample is either quick frozen, or immediately analyzed	lyophylization	Cu-Chelex 100 ion-exchange resin at pH 9.5 internal standard is used to check desalting efficiency	auto-analyzer using the nitrogen application technique	recovery depends on pH; optimal at pH 9.5; percentages range from 50% for acidic amino acids to 100% for the aromatic, basic, and neutral amino acids	1. Improved sensitivity is used to calculate correction factors for previous data. 2. The sample eluted from the desalting column is lyophylized and then transferred to the analyzer. 3. Sample is collected with a simple plastic bucket. 4. Authors use a "micro-column" after desalting to eliminate a nynhydrin-negative chromatography problem.
Starikova (1969) sea water and bottom sediments of Black Sea	14 free amino acids were identified; 14.6 - 45.7 μg/l. for free; and @5.8 - 14.8 μg/l. for combined amino acids were not reported	5 l. sample was filtered through a membrane filter (< 0.5μ) (type unknown covered with powered sand	evaporation of sample, and extraction with 80% EtOH. The residue was hydrolyzed	ion-exchange	paper chromatography; quantitativity by photocolorimetry; chromatographic spots were developed with ninhydrin	10% error in photocolorimetric procedure	1. The free amino acids included glycine, aspartic acid, ∝ - alanine serine, cystine
Jeffrey (1969) Gulf of Mexico approximately 25°N, 90°W	complex mixture of aldehydes, ketones, acids, esters, amines, aromatics, and aliphatic structures, conjugated carbohydrates, and amino acids	pre-combusted Gelman type A (0.3μ) fiberglass filters	fractionation on a silicic acid column followed by thin-layer chromatography	adsorption on activated charcoal eluted by light solvents	gas chromatography; mass spectrometer for carbon isotope composition	overall recovery of initial dissolved organic carbon was 60-80% and lower for coastal waters	1. Minor contamination was evident. 2. The adsorption column is incorporated into a continuous flow system. 3. Up to 3 gms. of organic matter was recovered from 9,000 l. of sea water

Table IV: Methods of Amino Acid Analysis (continued)

AUTHOR & WATER SAMPLED	COMPOUNOS FOUNO	FILTRATION METHOD	CONCENTRATION METHOD	OESALT METHOO	MEANS OF IOENTIFICATION	RECOVERY AND/OR SENSITIVITY	COMMENTS
Litchfield and Prescott (1970) Gulf of Mexico waters, and pond water	23 free amino acids with aspartic acid present in all samples and also arginine, glycine, and serine most prevalent; only qualitative results reported	500-5000 ml. samples were filtered through 0.45μ Millipore filters	amino acids are dansylated (5- dimethylamino-1-naphthalene sulphonyl chloride) and evaporated after extraction	diethyl ether extraction (four times)	2-dimensional TLC	30-40% recovery is claimed; 1 n-mole or less of a DNS-amino acid are detectable on TLC plates	1. Dansyl-derivatives are stable and have marked fluorescence. 2. Only applicable for free amino acids. 3. The dansylation reaction time is 20-24 hours. 4. There is some doubt whether the derivative is useful in sea water (Jeffrey and Webb, personal communication).
Bohling (1970) aged seawater samples from 54°N; 8°E	free amino acids, with glycine and serine, by far, the most prevalent	0.45μ membrane (type unknown filter and immediately frozen at -20°C.	evaporation to small volume	ion-exchange	Beckman amino acid analyzer, "Unichrom"	80% recovery and qood reproduction of results is claimed	1. Ion-exchanqe resins used proved to be a considerable source of contamination by ninhydrin positive compounds. 2. Based on Palmork (1963b). 3. Aspartic acid was difficult to isolate.
Riley and Segar (1970) Irish Sea at approximately (54°N, 5°W)	15 free and combined amino acids; acid, basic, aromatic, and neutral acids; acidic and basic acid concentrations were very low; 0.2 to 7.9μg/l. for free amino acids; 0.1 to 41.8μg/l. for combined amino acids	5-l. samples were passed through Whatman GF/C glass fiber filters covered with 0.5 mm layer of magnesium carbonate	evaporation <u>in vacuuo</u> in two stages (climbing film and rotary film evaporators)	same as Chau and Riley (1966) except Oowex 50 W resins were used for final desalting	quantitative determination of the 2-dimensional TLC spots by a Joyce Loeble Chromoscan	for 3-l. samples the maximum sensitivities by chromatography for the individual acids ranqed from 0.03 to 0.5μg/l. for 10-100 μl. of desalted amino acid solutions	1. This modified procedure required several hours less time of analysis; has qreater precision; sensitivity is doubled compared to Chau and Riley (1966)

Table IV: Methods of Amino Acid Analysis (continued)

AUTHOR & WATER SAMPLED	COMPOUNDS FOUND	FILTRATION METHOD	CONCENTRATION METHOD	DESALT METHOD	MEANS OF IDENTIFICATION	RECOVERY AND/OR SENSITIVITY	COMMENTS
Andrews and Williams (1971) English Channel waters	12 free amino acids; acid basic, neutral acids; no aromatic acids reported	7-l. sample was passed through Whatman GF/C glass fiber filter; hydrolyzed with 50% H_2SO_4; stored for two to five days at 5°C. before analysis	Chlex column eluate is evaporated, made acidic, and desalted again on cation exchange resin column	0.5 to 3 ml./ℓ. min. were passed through a Cu-Chlex-100 exchange column and eluted with either 3M-NH_4OH or 0.1M piperdine	Beckman or Technicon amino acid autoanalyzer to determine individual compounds; to determine total amino acids a photometric technique with ninhydrin was used as Stevens and Schinske (1961) worked out	not reported	1. Used radioactive amino acids to evaluate recoveries. 2. Found that the amount of distilled water (< 50 ml.) used to wash the Chlex column after organic retention is essential. 3. Other improvements on Siegel and Degens method (1966) are valuable.

complete, these are generalized by the flow diagram in Figure 2.

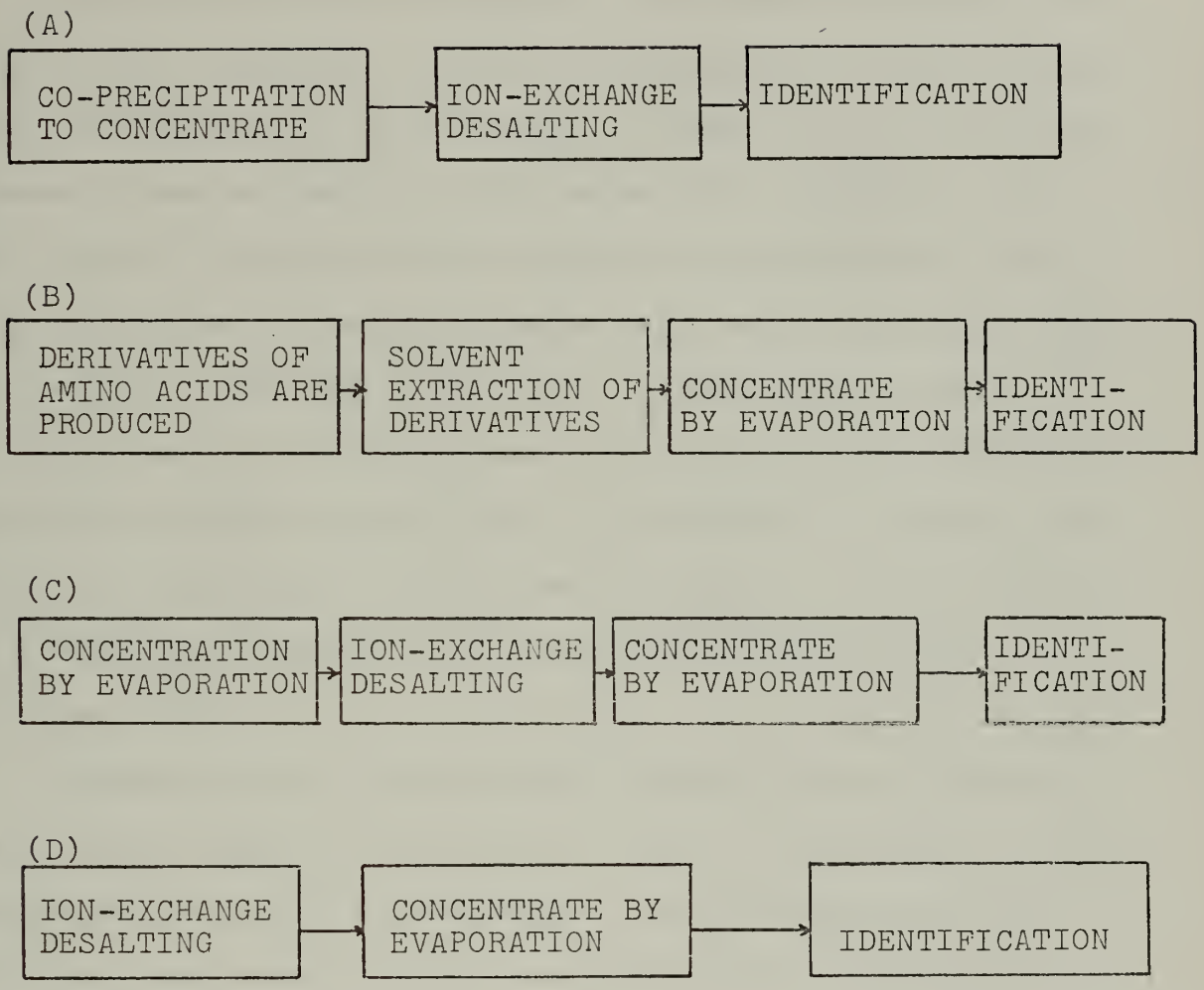


Figure 2. Flow Diagrams for Current Methods of Amino Acid Analysis

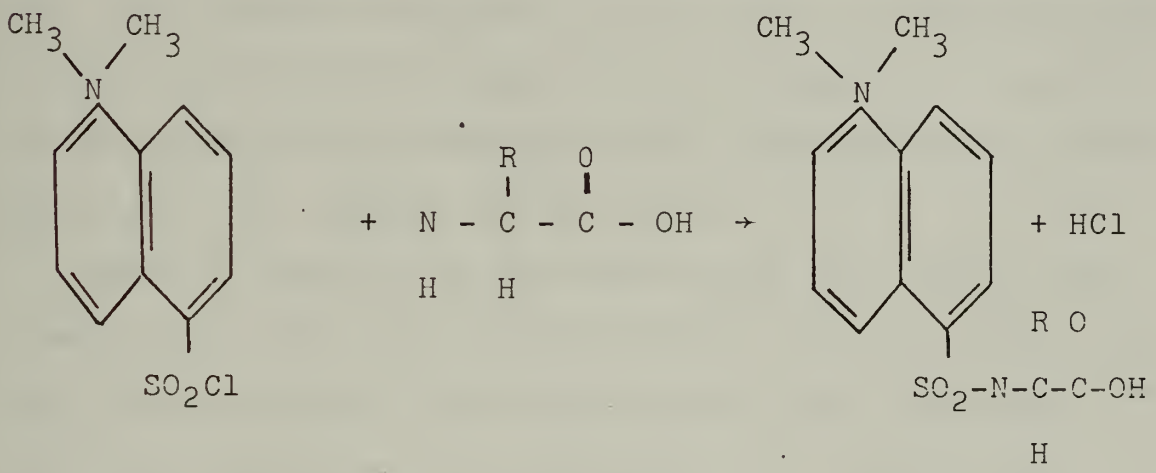
The details of each of these steps will follow later in this section.

The co-precipitation scheme A, developed at Texas A & M University, represents the first successful attempt to qualitatively determine the presence of amino acids in seawater. However, poor recovery of <50% under most conditions, and the long and laborious work involved to concentrate a substantial amount of organics makes this method less desirable than others [Chau and Riley 1966]. Commercial ferric chloride used to coprecipitate the organic matter introduces extraneous organic carbon which is difficult to avoid. And finally, especially for large water samples, it is difficult to remove the iron and other co-precipitated cations [Jeffrey 1969].

Scheme B, which processes the chemical derivatives of amino acids, has much potential. One nice advantage of this technique is that the derivatives can be manufactured in the field and preserved for analysis ashore, analogous to the Mm^{+2} addition step in the Winkler dissolved oxygen method. The 2, 4-dinitro-1-fluoro-benzene method [Palmork 1963a] seems to work better in natural seawater than does the recent dansylation method (1-dimethylaminonaphthalene-5-sulphonyl chloride) developed by Litchfield and Prescott (1970), [Webb and Jeffrey, personal communication]. However, the dinitrophenyl derivatives are unstable and light sensitive. Palmork reported only the recovery of neutral and aromatic acids, and Chau and Riley (1966) found poor recoveries for other

amino acids by this method. Suitable automation of the chromatography, after the dinitrophenyl derivate manufacture and its extraction, may develop this into a useful method.

Dansylation is the reaction of amino-terminal residues of proteins, peptides, or free amino acids with 1-dimethyl-aminonaphthalene-5-sulphonyl chloride (DSN-Cl) to form highly fluorescent amino acid derivatives.



DNS-Cl α-amino Compound "Fluorosphoric amino derivative"

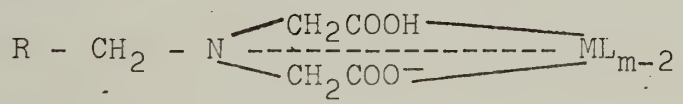
Although Dr. Webb found that dansylation did not work well in seawater, his examinations were not exhaustive. Because this process yields stable derivatives that are amenable to highly sensitive fluorescence spectrophotometric analysis it should be more extensively investigated in the future [E.D. Traganza, personal communication].

Scheme C, first proposed by Palmork (1963b) was employed by Chau and Riley (1966), who, after a careful and complete study of procedures proposed up to that time, recommended it

as "the most reliable method to determine amino acids." The salient feature of this scheme is that the sample is alternatively evaporated, while periodically removing salts from the mother liquor, and desalted with cation exchange resins. This method seems to work favorably and has been recently modified by Riley and Segar (1970) and is now claimed to be more precise, twice as sensitive, and less time consuming. The changes introduced by the latter investigators were the use of Dowex 50W exchange resins in place of Amberlite CG 120 resins in the desalting step, and a Chromascan [Joyce Loeble Co.] to identify the TLC spots.

Scheme D seems to be the method that most workers recommend in current work [Webb, Jeffrey, Harvey, Blumer, personal communication; Siegel and Degens 1966; Hobbie and Crawford 1988; Andrews and Williams 1971]. Amino acids are concentrated and isolated from seawater by ligand exchange on copper-Chelex 100 resin. Combined amino acids are determined by hydrolysis of the column eluate, which is then run through the ligand exchange procedure again to obtain them as dissolved free amino acids.

Chelex 100 resin (Bio-Rad Laboratories, Richmond, California) has extraordinary selectivities for transition metals. Such metals as copper will not bleed from the column when seawater is passed. The resin structure is



where:

M is the metal ion

N is the coordination number of the metal

L denotes the added ligand (e.g., amino acid) which becomes bound to the metal [Siegel and Degens 1966]

The active sites of the resin are the inodiacetic acid groups. The degree of success using this method is varied, which may be explained by variation in individual technique, and the varying quality of the resins. It is something of an art and a bit of luck in selecting a good batch of resin. Webb and Wood (1967) and Riley and Segar (1970) have examined Siegel's lead (1967) and worked out this method to a high degree of accuracy. Webb found that under his conditions, the method is least good for the acidic protein amino acids; it is usually poor for taurine, but is very good for the phosphonic acids that he has tried [Webb, personal communication].

Riley and Segar (1970) have found, using radioactive tracers, that maximum removal of amino acids occurred between pH 9.0 and 9.5. Appreciable loss of amino acids were found to occur if more than 50 ml. of distilled water is used to wash the column after desalting. Also, to remove traces of salt before introduction into the amino acid analyzer, the evaporated remains were passed through a cation exchanger.

The recovery efficiency of the Cu-Chelex column falls with continued use, and it must be regenerated. Webb and Wood, and Riley and Segar regenerate when the efficiency drops to 80%.

1. Evaporation Techniques for Concentration

Evaporation is the most common procedure to reduce the volume of samples before or after the desalting step. The fundamental criterion is to evaporate as gently as possible to prevent sample rearrangement or destruction. In order to minimize thermal decomposition, Palmork (1963b) evaporated the acidified sample under vacuum to about 20% of original volume in a climbing film evaporator. This evaporator heats small portions of the sample at a time, at moderate temperatures (≈ 50°c). The rotary film evaporator operates similarly and allows removal of salt crystals periodically to improve the efficiency [see Scheme C, in this section].

B. LIPIDS

There have been many investigations to isolate lipoid substances from seawater. This is essentially due to their higher concentrations relative to that of proteins and carbohydrates, and the fact that lipids are readily extracted by non-polar organic solvents.

Just as with amino acids and soluble sugars, a description of the sampler used is frequently absent in lipid studies. L.M. Jeffrey, in her extensive studies of lipoid

substances, recommends a polyvinyl chloride sampler rinsed with the extracting solvent. If quantitative results are desired, polyethylene samplers and containers should be avoided [Jeffrey 1970].

From the data in Table V, filtering is achieved, for the most part, by using 0.45 micron pore-membrane filters. However, no description of precautionary filter washing is evident. Garrett (1967), to avoid possible losses due to surface active material, did not filter his samplers at all.

1. Liquid Extraction

Riley and Skirrow (1965) concluded that solvent extraction is probably the most efficient method available for recovering dissolved organic matter from seawater for qualitative analysis. Since lipids lend themselves to this method, most investigators employ extraction. The most common system for lipid analysis after pre-filtering the sample includes solvent extraction with vacuum distillation, to remove the solvent, followed by either paper or gas chromatography.

There are several drawbacks to solvent extraction. These include contamination from impure commercial solvents, and compound degradation during multiple or prolonged extraction. Also, lipids tend to adsorb onto solid surfaces in acidic solutions and since acidic conditions are desirable during extraction, compounds may be lost. A pH level of about 2 was found to be optimum [Blumer 1970]. At higher pH, free acids are partly ionized and incompletely extracted.

Table V: Methods of Lipid Analysis

AUTHOR & WATER SAMPLED	COMPOUNDS FOUND	FILTRATION METHOD	CONCENTRATION METHOD	DESALT METHOD	MEANS OF IDENTIFICATION	RECOVERY AND/OR SENSITIVITY	COMMENTS
Koyma and Thompson (1959) and (1964) Northeast Pacific waters and inshore waters off Washington State	free acetic, formic lactic and glycolic acids with average offshore concentrations of 0.1 mg/l	20 l. sample was frozen on collection (-7°C) thawed, and filtered through Millipore filter	vacuum distillation, at 60°C., after extraction	continuous extraction in chloroform or ether at pH 3 for 3 to 5 weeks	partition chromatography on a silica column, eluted with chloroform containing increasing amounts of tert-butyl alcohol	recoveries of 95% to 100% attained after 3 weeks were confirmed by processing known concentrations; 85% recovery after one week	1. The greater part of these acids are presumably breakdown products during extraction. 2. Chloroform is easier to handle in continuous extraction systems. (Wagner 1969)
Slowey et. al. (1962) Gulf of Mexico approximately (26°N, 90°W) at 10 m. to 3000 m.	fatty acids of C-length 10-20 including mono- and di-unsaturated species at 0.1 to 0.8 mg/l	5 gallon samples through a 0.45μ pore-sized membrane Millipore filter; chloroform was added	concentration of extract by distillation under nitrogen at atmospheric pressure after extraction	extraction in ethyl acetate at pH 3	gas-liquid chromatography of methyl esters (method only permitted identification of C_{10} - C_{20})	90% extraction efficiency based on C-14 labelled stearic acid	1. The results indicate that this method was adequate for both qualatative and quantitative analysis of C_{10} and C_{20} acids. 2. Because the solvent is removed by distillation in a current of nitrogen, low molecular weight acids are lost. (Wagner 1969)
Jeffrey et. al. (1963), Jeffrey (1966) and (1968) Redfish Bay, Texas waters at 20 m. and Gulf of Mexico waters	hydrocarbons, glycerol esters of fatty acids, sterol esters, sterols, P- and N- compounds @ .4 to .8 $\frac{mg}{l}$	8 to 50 l. through a 0.45μ pored Millipore membrane filter	extract evaporated to dryness in a rotary evaporator	extraction in petroleum ether for steroids, and in ethyl acetate for other lipids at pH 2-3	fractionation by silicic acid column chromatography, gas chromatography, followed by infrared spectroscopic techniques to analyse each fraction	recovery 97% to 99%	Solvent extracts of sea water were separated into 8 lipid classes by solvents of increasing polarity.
Anita et. al. (1963, 1964)	glycolic acid 0.1 mg/l	0.45μ Millipore membrane filter	evaporation	———	photometric with 2.7 dihydroxy-naphthalene reagent	limit of detection was estimated to be 100 mgC/m³ per water as glycollic acid	Measurements were made investigating primary production in a plastic sphere
Degens et. al. (1964) Deep ocean water off Southern California (117°N, 32°W)	P-hydroxybenzoic, syringic and vanillic acids (1-3 μg/l.)	2 l. samples were frozen; after thaw, filtered through 0.45μ filter (type not described) under vacuum	filtrate was evaporated to dryness, acidified with HCl and evaporated again in vacuuo; phenols and indoles were extracted from dry salts with ethyl acetate	saline residue was first leached and finally desalted by ion-exchangers	two-dimensional ascending paper chromatography; diazotized para-nitroaniline was used as the spray reagent for phenols	reproducible to ± 10% confirmed by standards	Most phenolic compounds were found in hydrolyzates of the sea sediments

AUTHOR & WATER SAMPLED	COMPOUNDS FOUND	FILTRATION METHOD	CONCENTRATION METHOD	DESALT METHOD	MEANS OF IDENTIFICATION	RECOVERY AND/OR SENSITIVITY	COMMENTS
Williams (1961) and (1965) Sea water	six saturated and three unsaturated fatty acids, C_{12} to C_{22} (0.01 to .12 mg/l)	either through 0.5 μ filter or 2.0 μ glass fiber filters	evaporation	extraction with CCl_3, $CHCl_3$ and CS_2 at pH 2	gas chromatography of the methyl esters of the fatty acids	reproduced within 30%; minimum detectable amount was .01 mg/l; recoveries of 80.5 to 98.1% were confirmed	1. Concentrations were one or two magnitudes lower than previous reports for fatty acids. 2. C_{14} palmitic acid tracer was used to determine recovery. 3. A co-precipitation method was attempted but the chromatography was unsuccessful.
Kamatani and Matsudaira (1966) Seawater and sediments of undescribed origin	organic acids (formic, acetic, propionic, butyric, lactic, oxalic, tartaric, citric) @ 2 mg/l	1 l. through a 0.8 μ Millipore filter and adjusted filtrate to pH 9 to retain organic acids as non-volatile salts	evaporated @ 50°C. under reduced pressure	continuous ether extraction at pH 1-2 for seawater; distilled water extraction for sediments	silica gel chromatography using benzene-n-butyl alcohol (20%) as an eluting agent	>90% recovery for formic, acetic, butyric and lactic acids by chromatography	1. Organic acids may be decomposition products due to prolonged extraction. 2. Non-volatile acids were poorly recovered (< 60%)
Ushakov, et. al. (1966) Black Sea water (surface)	all saturated fatty acids from C_{11} to C_{20}; a few unsaturated C_{15} to C_{17} were also present; no concentrations reported	extraction was performed with ethyl acetate on 50 l. of water; then acidified to pH 3 and filtered 4 l. at a time through a "No. 5" membrane filter	each 4-l. portion was extracted 4 times with ethyl acetate at pH 3 and the extract evaporated to dryness in the vacuum of a water-jet pump	to completely free the residue of salts it was extracted with benzol and the solution evaporated to constant weight	gas chromatographic analysis of methyl esters fractionated on silica gel	95 mg. of organic matter were obtained from 50 l.	1. Claims this technique can be successfully applied in a systematic study of the concentration of organic acids in sea water
Garrett (1967) surface waters from Atlantic and Pacific Oceans, 8-200 miles from shore	fatty alcohols, fatty acids, fatty esters, and hydrocarbons @ 0.2 to 2.0 mg/l.	no filtration to avoid loss of surface active material by adsorption	co-precipitation with ferric chloride, chloroform added to concentrate; frozen	extraction with chloroform at pH 2, after sample thawed	gas chromatography of the methyl esters fractionated on silica gel columns	90-95% recovery was confirmed by examining standards	1. Found that the higher molecular weight and less water soluble fatty acids and alcohols are the more surface active. 2. Commercial $FeCl_3$ may contain organic contaminants. 3. Isolated organic fractions contain more than dissolved species.

Table V: Methods of Lipid Analysis (continued)

AUTHOR & WATER SAMPLED	COMPOUNDS FOUND	FILTRATION METHOD	CONCENTRATION METHOD	DESALT METHOD	MEANS OF IDENTIFICATION	RECOVERY AND/OR SENSITIVITY	COMMENTS
Swinnerton and Linnenbom (1965) and (1967) sea water was collected directly over bottom of Chesapeake Bay	low molecular weight hydrocarbons in the C_1 to C_4 range @ 10^{-4} ml/l	no filtration	purged gases were concentrated in cold water traps containing appropriate adsorbents	purging the sample with helium	sensitive gas-chromatography	absolute sensitivity is approximately $2X10^{-12}$ moles or approximately $5X10^{-8}$ ml dissolved gas per liter of sea water. At this lower limit, the precision of the method is 10%	1. This technique permits smaller samples, and very dilute solutions may be analyzed. 2. This method is applicable for hydrocarbons up to n-octane and also aromatics. 3. Ethane and ethylene were difficult to distinguish.
Adams and Richards (1968) anoxic waters (Nitinat Lake) and coastal waters	sterols and sterol esters, choline-containing lipids, and ninhydrin-positive compounds in both anoxic and O_2-bearing waters, whereas mercaptans were found only in anoxic areas (1.8 to 1.9 mg/l)	2.5 l. samples through either Gelman Type (0.3μ) filters or Millipore membrane filters (0.45μ) using slight Nitrogen overpressure	evaporated to constant weight in a nitrogen stream after extraction	extraction with petroleum ether and then ethyl acetate, at pH 2, for 2 hours in a rotary extractor	thin-layer chromatography	recovered 40-60% of the dissolved organic carbon with up to 87% in deeper waters, by comparison with standards	1. High values found may be due to: 1) use of ethyl acetate for extraction and 2) colder waters may have higher percentage of lipid materials than warmer waters. (Jeffrey 1968) 2. Meticulous measures are described to prevent contamination. 3. A nitrogen atmosphere was provided at all steps to avoid oxidation of volatile compounds.
Matthews and Smith (1968) Gulf of Mexico coastal waters	cholesterol, stigmasterol and sitosterol (10-135 mg/l)	through diatomaceous earth	silica gel chromatography	hexane extraction	infrared adsorption and gas chromatography of the free sterols and their acetate and dimethylsilyl derivatives	—	1. Plastic samples contributed contamination in the form of phtholates. 2. Sterols may have been extracted from planktonic material during filtration.

Table V: Methods of Lipid Analysis (continued)

AUTHOR & WATER SAMPLED	COMPOUNDS FOUND	FILTRATION METHOD	CONCENTRATION METHOD	DESALT METHOD	MEANS OF IDENTIFICATION	RECOVERY AND/OR SENSITIVITY	COMMENTS
Wallace and Wilson (1969) natural seawater	identification of proteins, fatty acids, polysaccharides, and possibly phospholipids and steroids in mixtures	membrane filter (kind and size not described)	foam separation tower	——	functional group tests, chromatography, and solvent affinity indicate classes of compounds found in the isolated mixtures	100% recovery of 5 mg/l of protein	1. Large volumes of samples can be processed in minutes. 2. Non-destructive. 3. Their foams were contaminated with detergents which weren't washed out. 4. Specific for surface active compounds. 5. Bubbled gas may be source of contamination. (Jeffrey, personal communication)
Riley and Taylor (1969) spiked sea water	n-heptanoic acid, n-heptadeconic acid, 4-ketoglutaric acid, cholesterol, various surfactants, insecticides, dyes, and humic acids @ 0.5 to $5X10^3$ mg/l. were adsorbed quantitatively	0.5 μ filters (type not described)	——	5 ml/min through Amberlite XAO-1 adsorption resins concentrate and desalt the filtered samples and eluted with KOH, NH_4OH, EtOH, HNO_3, where suitable	radiochemical techniques, photometric, fluorimetric and chromatographic methods	100% recovery confirmed	1. Most carbohydrates, amino acids, phenols, and proteins were not adsorbed. 2. Inorganic cations and anions were not adsorbed at all.
Blumer (1970) surface sea water	straight chained hydrocarbons, C_{14} to C_{33}; branched paraffins; isoprenoid hydrocarbons (e.g. pristane) olefinic hydrocarbons, fatty acids	Whatman #54 filter paper which has been Soxhlet extracted with benzene-methanol azeotrope	chromatography on silica gel into fractions	extraction in pentare at pH 2	column and gas chromatography, when possible. Chemical and spectral confirmation	not given	1. This work represents the first systematic approach to detecting all organic matter in seawater

At lower pH, the extracted material increases with extraction time due to non-extractable polymers or to the cell fragments that have passed through the filter [Blumer 1970].

The solvents used most extensively by researchers are ethyl acetate, chloroform, and petroleum ether. Pentane, hexane, and carbon tetrachloride have been used in some studies. Jeffrey (1970) has found that ethyl acetate and chloroform removed more dissolved organic material by weight, e.g., including substituted groups, than did petroleum ether. However, in most cases, petroleum ether was found to recover higher percentages of carbon. As a result petroleum ether is a desirable solvent for such species as hydrocarbons, sterols, fatty acids, and triglycerides - the less polar lipids. Chloroform and ethyl acetate are recommended for compounds with amino, hydroxyl, phosphate, or carboxylic acid groups.

Jeffrey (1970) found that ethyl acetate is more convenient than chloroform for extraction of seawater. However, as noted, acidic acid is formed when the seawater is acidified and must be removed before extraction and prior to further analysis. Also, besides having a higher blank than either petroleum ether or chloroform, ethyl acetate was found to dissolve more water and salt than both other solvents. This latter phenomena makes it difficult to dry the extracts and it also may yield erroneous concentration levels. Chloroform, on the other hand, is conveniently heavier than salt water, and does not form acetic acid nor dissolve appreciable salt. Dr. Jeffrey has also found that

for aerobic coastal and oceanic waters, chloroform is as efficient as ethyl acetate and much more efficient than petroleum ether for extraction.

In his systematic examination of all dissolved organic constituents in seawater, Blumer (1970) chose pentane as the extraction solvent for hydrocarbons and fatty acids. Dr. Blumer's choice is based on pentane's high volatility and ease of purification.

Decomposition is postulated to be a cause of erroneous concentration values after prolonged extractions [Kamatani and Matsudaira 1966]. If more than three extractions are required to recover a significant amount of lipids, another method of isolation should be used, since the compounds may be appreciably destroyed [Jeffrey, personal communication]. Blumer (1970) recommends mechanical agitation (Vibromixer) for qualitative results, and the use of separating funnels for quantitative investigations. An internal standard is a valuable tool to determine the recovery efficiency, and thus the usefulness of a particular solvent in recovering certain dissolved constituents. Williams (1961) and Slowey (1962) both used an isotopic tracer technique to do this. Slowey used C-14 labelled stearic acid which was assayed by a proportional counter to calculate the percent of recovery. Williams (1965) used a soluble tracer in the form of pentadeconic acid to examine the recovery of specific components. It should also be remembered that the efficiency of a

particular solvent will vary with the biological and chemical characteristics of the water sampled.

2. Lipoid Substances Measured and Other Isolation Procedures

Jeffrey (1963,1966,1968) has published the most comprehensive studies on lipids. In her studies, lipid extract was separated by silica gel chromatography into eight fractions, from the most polar, hydrocarbons, to the least polar, phospholipids. Most other researchers have focused on only one or two lipid species.

Swinnerton and Linnenbom (1965,1967) have developed a system to purge the volatile hydrocarbons from seawater. They have detected low molecular weight hydrocarbons up to n-octane. For higher molecular weight hydrocarbons, Blumer's (1970) pentane extract method is applicable.

Fatty acid detection has progressed through the efforts of Slowey, et al. (1962), Jeffrey (1970), Williams (1961,1965), Garrett (1967), and Ushakov, et al.(1966). These isolations were made, for the most part, by extraction although Garrett coprecipitated his sample with $FeCl_3$ before extraction. All but Ushakov subjected the total organic matter to extraction and followed by methylation and gas chromatography. Ushakov fractionated the fatty acid component first by thin layer chromatography before applying methylation and gas chromatography.

Other organic acids have been detected in seawater. These include phenolic acids [Degens, et al. 1964], short

chain organic acids [Koyma and Thompson 1959,1964], and volatile organic acids [Kamatani and Matsudaira 1966].

Sterols have been observed in studies by Adams (1968), Jeffrey (1970), Matthews and Smith (1968), and Riley and Taylor (1969). The latter paper introduced a new tool for isolating the dissolved organic components from seawater. These researchers used Amberlite polymeric adsorbents manufactured by Rohm and Haas Company, Philadelphia, Pennsylvania. These adsorbents are hard, insoluble polymeric spheres of variable surface area, porosities, and polarities. The non-polar adsorbents are particularly effective for adsorbing non-polar solutes from polar solvents. Conversely, the highly polar adsorbents are very effective for adsorbing polar solutes from non-polar solvents. The physical properties of Amberlite XAD adsorbents are summarized in Table VI. Using appropriate solvents, Riley and Taylor (1969) were successful in completely recovering a variety of compounds from seawater including fatty acids, sterols, vitamins, surfactants, dyes, insecticides, and humic acids. They used Amberlite XAD-1. Calder and Fritz (1970) at Ames Laboratory have used XAD-7 to isolate various lipids. They successfully recovered organic bases, carboxylic acids and phenols by eluting the Amberlite column with dilute strong acid (0.05 M HCl), dilute weak base (0.05 M $NaHCO_3$) and dilute strong base (0.05 M NaOH) respectively. Desorption was achieved by elution with ether, pentane, or methanol. After neutralization, they are readsorbed onto another column, stripped off, and characterized. Using a gas-chromatograph-mass spectrometer

TABLE VI

TABLE I TYPICAL PROPERTIES OF AMBERLITE POLYMERIC ADSORBENTS							
	Chemical Nature	Helium Porosity		Surface Area m^2 /gram	Average Pore Dia. Angstroms	Skeletal Density grams/cc	Nominal Mesh Sizes
		Volume %	cc/gram				
Nonpolar							
XAD-1	Polystyrene	37	0.69	100	200	1.06	20 to 50
XAD-2	Polystyrene	42	0.69	330	90	1.08	20 to 50
XAD-4	Polystyrene	51	0.99	750	50	1.09	20 to 50
Intermediate Polarity							
XAD-7	Acrylic Ester	55	1.08	450	80	1.25	20 to 50
XAD-8	Acrylic Ester	52	0.82	140	250	1.26	25 to 50
Polar							
XAD-9	Sulfoxide	45	0.61	70	360	1.27	20 to 50
XAD-11	Amide	41	0.62	70	350	1.21	16 to 50
XAD-12	Very Polar Nitrogen-Oxygen Group	45	0.79	25	1300	1.17	20 to 50
XE-284	Sulfonic Acid	47	0.66	600	40	1.44	20 to 50

NOTE: These are development products. Their physical characteristics are expected to conform closely to the values given above.

(from Rohm & Haas Publication IE 172 70)

combination, they were able to detect 42 compounds. The acrylic ester adsorbents (XAD-7 and XAD-8) are presently being used by several investigators at Woods Hole Oceanographic Institute to detect insecticide levels in pelagic waters.

Foam separation columns is another unique isolation tool now being developed at the Naval Research Laboratory by Wallace and Wilson (1969). Satisfactory progress is reported on fractionating such surface active materials as fatty acids, steroids, phospholipids, and proteins. The advantages of this method are that large volumes (≈7 liters) of seawater can be processed in minutes; degradation of compounds is minimal; and sample handling is reduced. The method is specific for surface-active compounds, and allows chemical and chromatographic identification of a significant group of dissolved organics in seawater. Jeffrey [personal communication] cautions against possible contamination from the foaming gas and recommends a larger foaming tower than the ones used by Wallace and Wilson (1969).

C. SOLUBLE SUGARS

Most methods to detect and identify soluble sugars in seawater have been developed for "total sugars." These techniques involve hydrolysis of the organic matter and spectrophotometric analysis of the resulting colored compounds. These colored compounds are the result of a reaction between the sugars and such reagents as n-ethyl carbazole, anthrone,

phenol-sulfuric acid, and orcinol-sulfuric acid. Such methods are commonly insensitive and non-specific.

Lewis and Rakestraw (1955) studied the total carbohydrate content in seawater by the use of the anthrone and n-ethyl carbazole methods. They found that, based on arabinose as a standard, both methods demonstrated comparable selectivity but observed that anthrone was the more sensitive of the two. Chloride-ion correction factors, and decomposition of the colored reaction product were problems. Guillard and Wangersky (1958) modified these methods by using glucose as the reference, and by placing a thin layer of mineral oil over the sample to prevent oxidation during the reaction.

The anthrone method was further evaluated for determinations of total hexose, keto- and aldo-hexoses, hexuronic acid, and pentose [Anita and Lee 1963]. These workers concluded that this was the most sensitive and precise colorimetric method to estimate these groups of carbohydrates. However, probable interference with other chemical constituents and a threshold sensitivity that bordered on the natural concentration levels of marine sugars were two serious drawbacks.

Dubois and his co-workers (1956) developed a phenol-sulfuric acid method to determine the presence of carbohydrates. In this method higher saccharides are hydrolyzed into monosaccharides and then cycled into derivatives of furfural. These derivatives are condensed with phenol to give a chromophore in solution which has an absorption

spectrum maximum in the visible region. The maximum absorption depends on the higher saccharides considered.

Handa (1966) made a comparative study of the phenol-sulfuric acid, anthrone, and n-ethyl carbazole methods. He concluded from his study that the phenol sulfuric acid method is the "most recommendable for determining total carbohydrate in seawater." Handa has used this method in extensive studies of the carbohydrate content of Japanese coastal and oceanic waters [Handa 1967a,1967b,1970].

1. Recent Improvements

Recently, efforts have been made to analyze and identify individual soluble sugars on a more continuous basis. Such tools as enzymatic assays, chromatographic columns, ion-exchange membrane electrodialysis, and automatic analysis systems have been employed in these more sophisticated systems. These methods are listed in Table VII.

2. Enzymatic Assays

Glucose, due to its relative abundance in the ocean (up to 45.6 μg/liter) [Josefsson 1970] and to its role in photosynthesis [Vaccaro, et al. 1968] is an important biochemical parameter to measure. Hicks and Carey (1968) have developed an enzymatic assay to determine glucose in seawater. After pre-filtration, glucose is coupled to a series reaction that yields a reduced coenzyme. Catalyzed by diaphorase the coenzyme is then allowed to reduce a dye, resazurin, to a highly fluorescent product, resarufin. The amount of

Table VII: Methods of Soluble Sugar Analysis

AUTHOR & WATER SAMPLED	COMPOUNDS FOUND	FILTRATION METHOD	CONCENTRATION METHOD	DESALT METHOD	MEANS OF IDENTIFICATION	RECOVERY AND/OR SENSITIVITY	COMMENTS
Wangersky (1952) inshore Gulf of Mexico waters	ascorbic acid and a "possible" rhamnoside" (0.1 g/l of rhamnoside)	not described	evaporation of eluate to crystalline compounds followed by separation according to solubility	activated charcoal adsorption column, eluted with EtOH	ultraviolet light adsorption; m-ethyl carbazole method	not reported	1. The amounts of ascorbic acid, as shown by the absorption spectra did not agree with the calculated amounts of carbohydrate found by the m-ethyl carbazole method
Schaefer (1964)	only attempted to isolate entire classes of compounds	not described	not described	ion retardation with Retardion 11A8	did not identify individual carbohydrates	author claims that 95-100% of carbohydrates are removed	1. Salts are adsorbed, and non-electrolytes pass through resin. 2. Joesefsson (1970) found that desalting was not completely effective (author claims 90% of salts are removed) 3. Resins need not be regenerated 4. Author could not completely remove sulfates from the sugars
Anita and Lee (1964) ocean water 5 to 3000 meters at (40°N; 143°W)	bound amino-sugars	Millipore membrane filter; filtrate was deep frozen	distillation of product formed by reagent	reaction of amino-sugar with a buffered acetylacetone reagent	spectro-photometrically with Ehrlich's reagent	sensitive to within 0.06 mg/l is claimed	1. No salt correction is needed 2. Little interference of color development from amino acids, or nutrients
Degens et. al. (1964) deep ocean water off southern California (117°N; 32°W)	glucose, mannose, galactose in the free state (14-35 μg/l.)	2 l. samples were frozen; after thaw, filtered through 0.45 μ filter (type not described) under vacuum	filtrate was evaporated to dryness, acidified to dryness and evaporated under vacuum; sugars were leached from dried salts by 80% EtOH	final desalting was by cation exchange resins for the dissolved residue.	one-dimensional descending paper chromatography	claim ±15% precision based on visual comparison of standards on the same sheet	1. Sugars were isolated on the paper by a 4% solution of tri-phenyl-tetrazolium chloride

Table VII: Methods of Soluble Sugar Analysis (continued)

AUTHOR & WATER SAMPLED	COMPOUNDS FOUND	FILTRATION METHOD	CONCENTRATION METHOD	DESALT METHOD	MEANS OF IDENTIFICATION	RECOVERY AND/OR SENSITIVITY	COMMENTS
Schaefer (1965) seawater	rhamnose, ribose, and sucrose	not described	not described	adsorption on carbon-celite columns, eluted with 20% EtOH	analytical micro-methods of eluted sugar fraction	not reported	Author has worked out the optimum conditions for isolation of soluble sugars
Hicks and Carey (1968) inshore seawater	Glucose (3.6 to 10.8 $\mu g/l$)	250 ml. sample passed through a 0.45μ Millipore filter under a 10 cm. head of water	———	enzymatic essay	fluorescent essay product, proportional to glucose, is measured with fluorometer	sensitivity of 3×10^{-8} M glucose, or 3 $\mu g/l$. of glucose	1. Seawater samples must be diluted with 2 volumes of distilled water. 2. Results were confirmed independently by a bioassay method (Vaccaro and Jannasch, 1966).
Keiling (1968) Atlantic ocean water (50°N; 14°W) @ 5-200 m.	glucose, galactose, saccharose, maltose, raffinose	1μ nylon Millipore NPNP filter	dialysis is used to separate the colloidal from the dissolved state after filtration	adsorption on activated charcoal eluted with 10% ethanol	the optical densities of the eluted sugars are compared with standards	90% adsorption efficiency is claimed	The author employed filtration on nylon, dialysis, and adsorption on carbon, to isolate the particulate, colloidal, and dissolved carbohydrates respectively.
Josefsson (1970) coastal waters	rhamnose, ribose, arabinose, xylose, fructose, mannose, galactose, glucose (0.15 to 45.6 $\mu g/l$)	HgCl was added, then filtered with 0.45μ Millipore filter	evaporation under vacuum	electrodialysis with an ion-exchange membrane	partition chromatography on an anion, and/or a cation exchanger; auto-analyzer using orcinol method	10% percentage error is claimed	1. Ethylene glycol, glycerol and sucrose were possibly present. 2. Gas chromatography was compared in the present study to partion chromatography and found inferior. 3. Desalting time may be reduced from the 24-30 hours under this study's conditions.
Andrews and Williams (1971) English channel (50°N; 04°W)	glucose (1-10 $\mu g/l$)	precombusted Whatman GF/C glass fiber filter; acidified; stored at 5°C	rotary evaporation at < 40°C; lyophilized	adsorption on charcoal-celite columns eluted with 10% EtOH	enzymatic glucose-oxidase system	recovery and sensitivity were checked by C^{14}-labelled glucose; 1 $\mu g/l$ sensitivity is claimed	1. A brownish material eluted with the sugars and interfered with analysis. 2. Humic substances prevented silyl derivatives to be manufactured for gas chromatographic determination.

resarufin produced is proportional to the amount of glucose present in the original sample. A fluorometer was used to measure the excitation and emission peaks of resarufin, which after calibration yielded the amount of glucose present in their 3 milliliter water samples. By comparison with standards, they found the assay to be sensitive to within 3 μg/liter. Their results were confirmed independently by Vaccaro and Jannasch (1966). Vaccaro, and his co-workers (1968) have successfully employed this assay to demonstrate the occurrence and role of glucose in the open ocean.

In a recent paper, Andrews and Williams (1971) measured the oxidation rate and concentrations of glucose in the English Channel. In their method the sugars were isolated on a carbon adsorption column, eluted, and then determined enzymatically by the glucose-oxidase system. Based on C_{14}-labelled glucose as a tracer, they claim a sensitivity of within 1 μg/liter of glucose. With concentrations of carbohydrates commonly within the range of 1-10 μg/liter, the precision associated with such enzymatic assays are not assuring. Because of the high selectivity characteristic of enzymatic assays, and their elimination of any isolation and fractionation steps, more sensitive assays would be attractive.

3. Other Techniques

Several investigators have been successful in isolating sugars from seawater by using charcoal absorption columns [Wangersky 1952; Schaefer1965; and Keiling 1968].

Desorption from the column, always a problem with carbon absorption, has been achieved by gradient elution with 10-20% ethanol. Improved results have been obtained by pre-treating the carbon columns with Celite (hyflo-supercel) [Keiling 1968]. Schaefer (1965) has worked out a set of optimum conditions and details for the isolation of soluble sugars from seawater.

Electrodialysis using ion-exchange membranes has been used by Josefsson (1970) to isolate soluble sugars from sea waters. This method has great potential for neutral molecules like sugars, which are not greatly influenced by an electrical field. Because desalting is continuously carried out electrically, the membranes do not require any chemical regeneration. A 2.2 liter capacity electrodialysis unit has been used by Josefsson. The membranes themselves are manufactured by milling ion-exchanger beads onto a binder such as polythene. An anionic and a cationic selective membrane are used simultaneously. Although the desalting time is 24-30 hours, Josefsson claims that this time may be significantly reduced without loss of efficiency.

VI. IDENTIFICATION OF INDIVIDUAL SPECIES

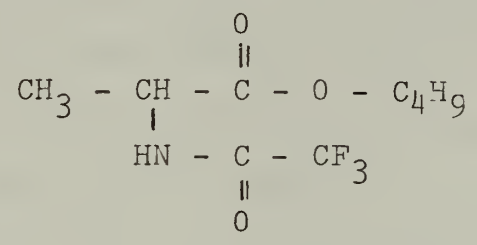
A. AMINO ACIDS

From Table IV, it can be seen that there are basically four techniques that are used to identify the individual amino acids or their derivatives in a desalted seawater

sample. These are two dimensional paper chromatography, two dimensional thin-layer chromatography, gas chromatography, and an automatic amino acid analyzer.

In a comparative study, Chau and Riley (1966) found that thin-layer chromatography is more sensitive and rapid, and the resolution of the amino acids is better, and the tailing is less, than in paper chromatography. Jeffrey [personal communication] recommends spraying with 0.1% ninhydrin in n-butanol or acetone, and heating at 110°C for 15-20 minutes. In addition, the spots can be easily removed and eluted for spectrophotometric analysis. However, this method can only give partially quantitative data at best. Degens, et al. (1964) made visual comparisons of TLC spots with knowns and could only be accurate within ± 15%. Quantitative results are increased by using an analytical scanning device to examine spots representing the ninhydrin complexes. Riley and Segar (1970) used the Joyce Loeble Chromoscan with a thin layer scanner attachment. They found this to reduce their analysis time by several hours, and produced maximum precisions from ±0.03 μg/l for alanine to ±0.5 μg/l for phenylanaline. The eluate from a Cu-Chelex 100 column was found to contain a ninhydrin-negative material chromatographing in the non-leucine region [Wainer and King 1965]. Webb and Wood (1967) have seemed to solve this problem by passing the eluate from the Cu-Chelex column through a "micro-column" of Chelex 100 in the NH_4^+ form. Their results indicate complete purification.

Gas chromatographic procedures, for natural amino acids [Gehrke, et al. 1968] and Kunisaki, et al. 1969] have been developed recently. These researchers found that n-trifluro-acetyl n-butyl esters to be the amino acid derivative best suited for gas chromatographic analysis.



N-trifluoroacetyl, N-butyl ester of alanine

The particular advantage of these esters lies in the fact that three fluorine atoms are introduced into the molecule, increasing sensitivity to electron capture detection. Kunisaki and his co-workers (1969) found that except for histidine and cystine, tryptophan and arginine, the reproducibility was very good for all other amino acids.

The amino acid identification tool used more and more, and recommended by many workers, is the automatic amino acid analyzer. This device allows quantitative determination of amino acids by automatically recording the ninhydrin color value from the effluent of ion-exchange columns. Ion-exchange resins are used as the separation medium. Technicon and Beckman instruments have been used. With this analyzer, the hydrolyzate of a protein or peptide may be analyzed in less than 24 hours; with minimum attention. An advantage of such continuous autoanalyzers includes less

manipulation of the sample during the automated process. However, the problems of collection, filtering, desalting, and concentration remain.

B. LIPIDS

Based on the many studies and observations by Jeffrey (1963,1966,1970), the seawater-solvent extracts can be characterized both qualitatively and quantitatively.

Complete quantitative results are time-consuming, requiring separation of the lipid extract by elution from a silicic acid column, thin-layer chromatography of the fractions obtained, and chromatography of the lipid derivatives. In some cases, mass spectrometry is necessary after chemical separation for absolute identification. Jeffrey was able to separate the lipid component into 8 fractions according to their polarity. The silicic acid columns used by Jeffrey were eluted in the following order, with 1%,4%, and 25% ethyl ether in petroleum ether; pure ethyl ether; acetone; 20% and 50% methanol in dichloromethane; and pure methanol. This elution scheme separated the 8 fractions. Blumer (1970) describes another scheme used in his pentane extract study. He also used silica gel and notes that the adsorbent should be partially deactivated to minimize catalytic reactions such as dehydration. Functional group tests [Wallace and Wilson 1969], solubility tests [Jeffrey 1963] and photometric techniques [Anita 1963; Riley and Taylor 1969] are applicable

to such isolated fractions. These auxiliary tests are primarily used to confirm thin-layer or gas chromatography analyses.

Qualitative results can be obtained by thin-layer chromatography of the lipid extract of 10-20 liters of water. Jeffrey [personal communication] recommends chromatography with Silica Gel G on 8" x 8" plates in 1) Toluene-ethyl acetate (19:1 by volume) for hydrocarbons, sterols, fatty acids, etc. (non-polar compounds); 2) chloroform-methanol-water (14:1) for increasingly polar compounds (Phenols, simple substituted phenols); 3) Butanol-acetic acid-water (4:1:1) for even more polar compounds and finally, 4) Pyridine-acetone-NH_4OH(10:6:5). Spraying with 0.2% 2,7 dichlorofluorescein in methanol shows up lipids very vividly under an ultraviolet light (long wave length). No heating is necessary for development of the spots.

Gas chromatography has been used to identify lipoid substances by various workers [Jeffrey 1963,1966,1970; Slowey 1962; Williams 1961,1965; Swinnerton and Linnenbom 1967; Ushakov, et al. 1966; Garrett 1967 and Blumer 1970]. This analysis has been applied to either the raw solvent extracts or to the methyl derivatives of the constituents of the fractions eluted from chromatographic columns, and thin-layer chromatographic spots.

C. SOLUBLE SUGARS

For the most part, final identification of soluble sugars has been attempted by analysis of enzymatic reaction products, spectrophotometric techniques, and simple analytical micro-methods [Table IV]. These methods have either been too insensitive to lower concentrations, or subject to excessive manipulation of chemical reagents while attempting to effect a desired reaction. Non-reproducibility and low resolution of the total sugar content into its individual species are also shortcomings. However, a new procedure, worked out by Mopper and Degens (1971) has minimized these problems, and is developed for use in an auto-analyzer system [Technicon 1965].

Mopper and Degens pass the sample continuously through an anionic column in the sulfate form. The sugars are separated in the column as it is eluted with 89% EtOH. The eluted sugars are treated continuously with a basic solution of tetrazoleum blue. Tetrazoleum blue is more sensitive and less corrosive than either orcinol-sulfuric acid, anthrone or phenol-sulfuric acid. The limit of sensitivity is 10^{-8} to 10^{-9} moles. The major success of this method is due to the development of the applicability of tetrazoleum blue for use in capillary tubing. Monosaccharide mixtures are fully resolved in 3-4 hours. This procedure should be able to complement Josefsson's electrodialysis procedure to give quantitative results for soluble sugars.

VII. DISCUSSION AND CONCLUSIONS

The primary objective of examining the chemistry and techniques used in the study of trace organic matter in seawater was to sort out methods of rapid analysis that can be used routinely by chemical oceanographers on board ship. Other criteria considered in this analysis include:

1. Selectivity for specific type of molecules, with respect to size, functional groups, etc.;
2. Destructive or non-destructive analysis with respect to compound alteration;
3. Continuous, or batch in operation;
4. Quantitative or qualitative analysis;
5. Reliability based on usage by independent researchers;
6. Rigorous, or quick analysis for approximate results with respect to quality and accuracy of the desired data.

One finding that is apparent is that there is no procedure standardization. This is evident especially in sampling and filtration. Because of this variability from technique to technique, it was extremely difficult to compare the results of independent researchers. Much of the published works on dissolved organics is qualitative or unreliable due to the use of unsatisfactory or incompletely tested analytical methods. Chau and Riley (1966) arrived at this same conclusion, but their findings were not emphasized, and research

efforts, based on questionable processes continue to ramify. This report demonstrates the diverse procedures used, and should help to direct future efforts in a direction that will achieve routine, rapid, reliable techniques which can be used as tools of chemical oceanography.

The first analytical consideration must be the collection of representative samples of seawater from the water column. Table III lists samplers that have been used or recommended for collection of seawater for dissolved organic analysis. Samplers collect seawater in discrete volumes *in situ* or by pumping from depth to a vessel at the surface. Present analysis systems require both kinds of raw seawater input. They are each useful with appropriate systems.

An ideal *in situ* or batch type sampling device has been developed by Clark and his co-workers (1968). Their "rupture disc-triggered" sampler is specifically designed for dissolved organic work, and it ensures minimum contamination by using glass. Although others recommended polyvinyl chloride there is always some quesiion about bleeding plasticizers and the problem of keeping this material clean. Clark's device is worthy of consideration as a standard for obtaining synoptic dissolved organic profiles. Similar "rupture disc" bottles should be designed which can be attached in multiples on a chemically inert hydrographic "wire." Uncontaminated, representative seawater samples must be collected if analysis of trace organics is to become a routine matter.

Pumping systems, constructed of noncontaminating materials, such as those developed by Zeitoun and his co-workers (1965) have the advantage of collecting continuous samples which have the potential for obtaining continuous profiles as well as large volumes. There is still some doubt as to possible mixing, compounds alteration, or biological cell rupture during passage through the tubing and the pump itself. A feature which may be developed for pumping seawater from various depths is on line filtration, preferably at depth. Some attempt has been made by Laird (1967) as well as Zeitoun (1965). In the past, continuous pumping systems have been used for delivering large volumes to collecting systems epitomized by the charcoal adsorption columns used by Jeffrey (1969). While Jeffrey's objective was to obtain large amounts of organic material for identification, systems like this may have a future when more sensitive systems which require less water are available.

The results and observations of many workers suggest that combusted glass fiber and washed metal fiber filters be used in conjunction with an inverted multiple filter system. Pre-centrifugation may be useful with the development of a large volume centrifugation unit. The main advantage of glass and metal fiber filters over cellulose ester membrane filters is more effective filter cleansing. Means should be developed to use glass and metal filters "in line" with continuous pumping systems, as well as in batch filtration. The inverted multiple filtration principle used by

Lewis and Traganza (1971) minimizes filter clogging and compound and cellular alteration. Parker (1967a) and Lewis and Traganza (1971) both concluded that use of large volume centrifugation as a pre-filtration step to remove biological cells that might rupture on, or clog the filter, is the most efficient separation scheme. Ideally, a simple and effective filtering procedure should be agreed upon for widespread use. In this manner, future studies would have a more representative "dissolved organic fraction" upon which to begin analysis, and compare independent results.

After proper "filtration," the seawater filtrate is assumed to contain organic and inorganic matter no larger than 0.45 microns in diameter. Isolation of the dissolved organic matter in this sub-sample from the relatively vast amount of inorganic salts (desalting), and subsequent fractionation into individual organic compounds, are both essential and formidable steps in the analytical scheme. It is not a simple task to sort out accurate and reliable methods that are applicable to routine use aboard ship.

The results of this systems analysis of the methods for analyzing the three groups of dissolved organic compounds selected for consideration - amino acids, certain lipids, and soluble sugars - demonstrate that current methods employ a variety of techniques. After analysis and systematic evaluation of all these techniques, those which would serve as rapid and potentially routine tools for trace organic work were selected.

Based on the data in Table IV and the recommendations of many investigators, free and combined amino acid concentrations can best be measured by the use of a Cu-Chelex 100 resin column to selectively adsorb and remove the amino acids from the dissolved salts. This step is followed by elution, concentration by film evaporation, and identification adapted to autoanalysis of the concentrate. Andrews and Williams (1971) have successfully worked out the latest details of this scheme. The weakest link in this system is in the inaccuracy during autoanalysis of individual amino acids caused by chromatographic interference. The use of an identification scheme developed by Gehrke (1968) and Kunisaki (1969) and their co-workers would obviate this shortcoming by forming an amino acid ester that is amenable to sensitive gas chromatography. These authors report that n-trifluoroacetyl n-butyl ester derivatives allow excellent precision with most free and bound amino acids. With appropriate automated gas chromatography as an improvement over the autoanalyzer [Figure 3], this should be a reasonably rapid and routine technique that could be taken to sea.

Other methods of amino acid analysis include the derivative reaction techniques developed by Palmork (1963a) and Litchfield and Prescott (1969). The potential of these methods lies in the fact that they minimize compound alteration by processing amino acid derivatives that are manufactured in the raw seawater sample. The dansylation reaction, which yields a highly fluorescent product, is desirable due to the high

sensitivity of fluorescence analysis. However, until such problems as reaction rate, low selectivity and recovery are solved, these derivative techniques are not now applicable.

The majority of the research on lipid analysis [Table V] has been based on liquid extraction. Although this technique is simple, it is not attractive due to the time consuming multiple extractions required and the cumbersome handling involved. A recently developed adsorbent technique is superior in this respect, and has successfully isolated fatty acids, sterols, vitamins, surfactants, dyes, insecticides, humic acids, phenols, and organic bases from sea salts [Riley and Taylor, 1969; and Calder and Fritz, 1970]. Both research teams used Rohm and Haas Amberlite adsorbents. Since the adsorbed species must be eluted from the column in sequence according to their polarity, after neutralization, the eluted sample is readsorbed onto a second Amberlite column, stripped off, and characterized by gas-chromatography [Figure 3]. Such an analytical arrangement could be set up in a shipboard laboratory to analyze these important organic constituents at sea. Reagents, a few adsorption columns, and a gas chromatograph would be all the materials that are needed to routinely and selectively measure fatty acids, phenols, and sterols from this broad spectrum of compounds.

Light hydrocarbons have been successfully assayed by a method developed by Swinnerton and Linnenbom (1967). This method is extremely accurate due to highly developed gas chromatography. In addition, Swinnerton has refined his

system to a relatively rapid, and routine analysis of hydrocarbons up to n-octane. For heavier hydrocarbons Blumer's pentane extraction method is reliable , but suffers from the shortcomings of other liquid extraction techniques.

Foam separatory columns are used to isolate surface active lipids and proteins. This technique which is currently being developed at the Naval Research Laboratory may become a useful tool in some analyses, but is not very practical for general shipboard use.

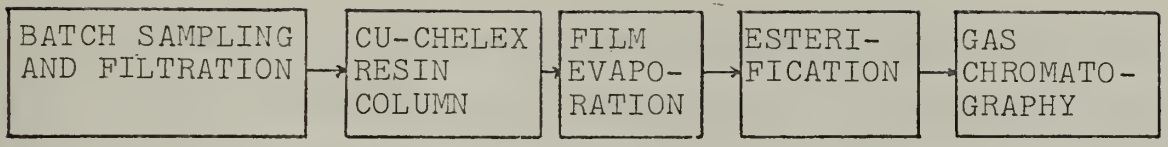
Because "total sugar" methods are not selective, and enzymatic assays are not sensitive enough, most investigators measure soluble sugar concentrations utilizing charcoal adsorption columns. Continuous or batch sampling is applicable to carbon columns, but of course the adsorbed sugars must be eluted. Successful desorption of the sugars has been achieved by gradient elution with 10-20% ethanol [Keiling 1968]. Other advances include pre-treatment of the carbon with Celite, a hyflo-supercel, to accelerate flow through the column. Such columns, seven feet in length, have been successfully used by Jeffrey (1969) to isolate three milligrams of organic matter from 9,000 liters of seawater pumped from depth. The unattractive feature is the time consuming desorption. However, once the raw seawater is pumped through the columns, they may be sealed for later elution and fractionation. The newly developed method of Mopper and Degens (1971) allows identification of the desorbed sugars on an

autoanalyzer, after evaporation in a climbing or rotary film evaporator [Figure 3].

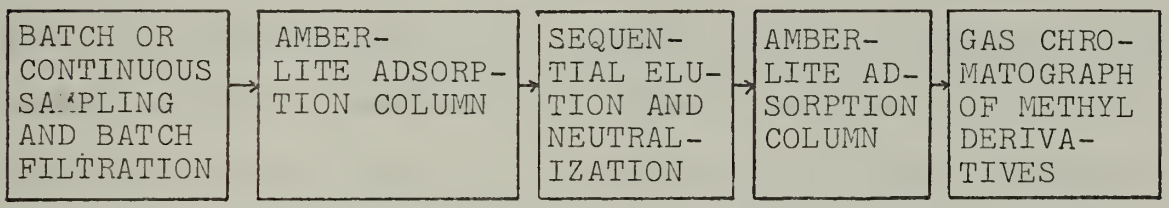
Ion-exchange membrane electrodialysis first proposed by Jeffrey and Hood (1958) has recently been developed to a high degree of accuracy by Josefsson (1970). In this method, 2.2 liters at a time are electrodialyzed through an anionic and cationic membrane simultaneously. This desalting step may be effected in several hours with sufficient voltage. However, this is not reasonably rapid for shipboard use. The Mopper and Degens autoanalyzer method (1971) is also applicable to the desalted seawater after film evaporation.

Figure 3 summarizes systems which are considered potentially the most rapid and accurate methods for measuring trace concentration levels of amino acids, lipids, and soluble sugars in seawater. Batch type samples collected by modified "rupture disc" bottles are proposed in a normal hydrocast mode. Large volume pre-filtration centrifugation and/or reverse flow multiple filtration are proposed as procedures for removal of the undissolved fraction. Soluble sugar analysis is patterned after the semicontinuous charcoal scheme developed by Jeffrey (1969).

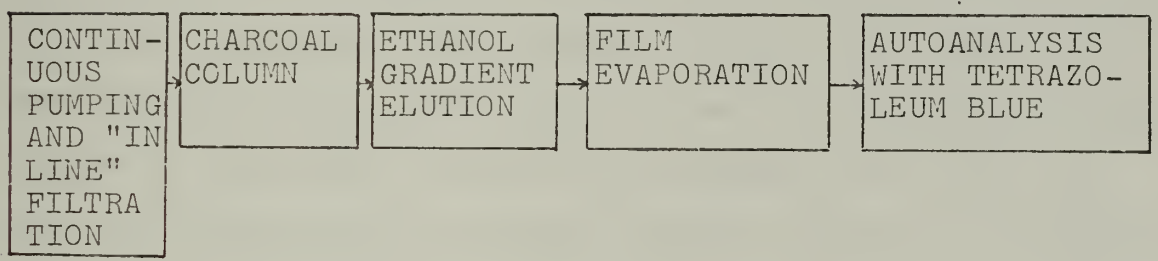
Once these systems for measuring these organic compounds are a reality, they can be complemented with Edhardt's (1969) ultraviolet autoanalyzer technique for measuring total organic carbon. Further useful correlations may be obtained by measuring urea concentrations which are apparently already a routine matter with the use of the autoanalyzer [Newell 1967].



AMINO ACID ANALYSIS SYSTEM


BATCH OR CONTINUOUS SAMPLING AND BATCH FILTRATION
AMBER-LITE ADSORP-TION COLUMN
SEQUEN-TIAL ELU-TION AND NEUTRAL-IZATION
AMBER-LITE AD-SORPTION COLUMN
GAS CHRO-MATOGRAPH OF METHYL DERIVA-TIVES

LIPID ANALYSIS SYSTEM



SOLUBLE SUGAR ANALYSIS SYSTEM

Figure 3. "Ideal" Analytical Schemes for Measuring Dissolved Organics in Seawater

The results of this study have unquestionably supported gas chromatography and the "autoanalyzer" as powerful tools which are beginning to solve some of the difficult analytical problems of the organic chemical oceanographer. There are more refinements to be made, especially in autoanalysis of specific organic compounds. Perhaps some professional society should sponsor a conference on organic systems. This paper could be a useful basis for organizing this type of effort.

So many physical, biological, geological, and chemical processes and properties are influenced by dissolved organic matter that it is imperative that new analytical methods be developed and used. For example, glucose and glycine are virtually ubiquitous and should be able to indicate biological trends in the ocean. These two compounds are "ideal" in that they are readily measured, highly variable, and widely occurring. Routine measurements of such compounds may reveal or predict many marine processes. We may not be able to discover the entire spectrum of dissolved organic matter before it has changed significantly, but wide analytical coverage of key compounds may provide an adequate index.

Future plans should be to develop and use automated analytical systems similar to those described in this paper. If these systems can be developed and coupled with "autoanalysis" of total organic carbon, it would be possible to construct a complete synoptic picture of the importance of dissolved organic matter in oceanic processes and properties of seawater.

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13. ABSTRACT

The diffuse field of marine analytical organic chemistry is systematically analyzed to select schemes for measuring the trace amounts of dissolved organic compounds which can be adopted as standard, rapid, routine tools to advance the oceanographic understanding of this important aspect of the ocean.

The many methods considered for measuring dissolved organic compounds in seawater have been systematically reduced to three systems that are potentially routine and rapid for shipboard work. These include quantitative gas chromatographic analysis of all amino acids and qualitative results for histidine, cystine, tryptophan, and arginine; gas chromatographic analysis of lipids including light hydrocarbons, fatty acids, and sterols, and; autoanalysis with tetrazoleum blue after charcoal adsorption for soluble sugar compounds like glucose, sucrose, and fructose.

Pumping systems or glass samplers on a chemically inert hydrographic wire followed by pre-centrifugation and inverse multiple filtration in combination with a selected analytical scheme will provide a system for routine analysis of seawater. Synoptic data of these compounds can be compared with total organic carbon which is currently the only routine analysis for organics in seawater.

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